The behavioural responses of sheep to the food deprivation associated with transport

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To my parents

Contents

Declaration

This thesis is my own composition, all references and assistance from other individuals have been acknowledged. The work in chapters 2 and 7 is entirely my own. The experimental work in chapters 3, 4, 5 and 6 was part of a larger project funded by the Scottish Office, Agriculture and Fisheries Department, and other workers were involved in the conduct of these experiements. The author had no input in the original grant application, but was involved in the setting up of the experiments, and in the data collection reported in this thesis. For chapters 4, 5 and 6, the author set up the computer system used to record the behaviour, and trained the other observers working on the project to use it. Blood samples were analysed by other members of the group. All data presented in chapters 3, 4, 5 and 6 were analysed independently of the group by the author and the chapters were written independently of publications arising from the larger project.

R. E. Jackson, 21st May 1999

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Abstract

Until recently, little has been known about the potential effects of transport on the welfare of sheep. One important factor in the transportation process is the withholding of food for long periods of time. This program of work was undertaken to obtain information on the effect of food deprivation on sheep in terms of changes in behaviour, blood biochemistry and feeding motivation.

Chapter 2 investigates the behavioural responses of sheep to food deprivation of up to 24h. Changes in behaviour during deprivation suggest that sheep were actively seeking food.

Chapter 3 investigated the effect of elevated temperature on the behaviour and physiology of sheep deprived of food for 24h at space allowances similar to those used for the commercial transportation of sheep. Evidence of fasting was found during treatment. Post-treatment there was an increase in feeding and evidence that the sheep became dehydrated as they fed.

Chapters 4, 5 and 6 investigated three aspects of transport, the provision of a period of lairage during a journey, and the effects of familiarity of post-transport housing and an additional period of food deprivation following a journey on the behavioural responses of sheep. The provision of a period of lairage facilitated post-treatment recovery, reducing the changes in liveweight during the treatment period and shortening the length of time for behaviour to return to pre-treatment levels. Changes in diet and environment post-transport were found to affect the behaviour of the sheep. Withholding food after 15h transport was associated with an increase in food-seeking behaviour which decreased after food was provided.

Chapter 7 investigated the feeding motivation of sheep after 6h-24h without food. There was an increase in feeding motivation after 6h without food which was reduced by providing food.

CHAPTER 1 Introduction

There are over 43 million sheep in the UK (compared with about 12 million cattle and 8 million pigs), with an annual output of over £1,300 million (Central Statistical Office, 1998).

In the UK, approximately 16 million sheep per year are transported to slaughterhouses by road (Warriss *et al.*, 1990), and until recently live exports to Europe have been increasing. Problems with transporting livestock have been found from both an economic and welfare viewpoint, with injury to animals resulting in damage to carcasses (Hails, 1978). More rarely, death can occur, a mortality rate of 0·018% has been reported for sheep transported by road in the UK (Knowles *et al.*, 1994a), increasing to 2·2% in sheep transported from Australia to the Middle East by ship (Norris & Richards, 1989). The mortality rate for sheep obtained from auction is higher (0·031%) than among sheep obtained direct from a farm (0·0068%) (Knowles *et al.*, 1994a).

Legislation governing the welfare of farm animals during transport has changed a number of times in the last decade. Only the parts of the legislation concerning provision of food and water will be considered here, these being of most relevance to this thesis. Legislation in the UK in the early 1990s required the provision of water, food and rest at least every 15h during transport (The Welfare of Animals During Transport Order, 1992). Although a revised Order was introduced in 1994 (The Welfare of Animals during Transport Order, 1994), the requirements for the provision of water, food and rest remained unchanged. As work has demonstrated that the majority of journeys within the UK are less than 10h in

duration (Warriss *et al.*, 1990), longer journey times occur when sheep are exported to mainland Europe. The number of sheep being exported to mainland Europe increased dramatically in the early 1990's. In 1992, 1.3 million sheep were exported to mainland Europe, reaching a peak of 1.9 million in 1993 (MLC, 1994). Numbers have fallen again, with an estimated 440,000 sheep exported in 1997 (MLC, 1998). The implementation in the UK of EC Directive 91/628 as The Welfare of Animals (Transport) Order (1997) sets maximum journey times of 8h. If the transporting vehicle meets certain requirements this journey time can be increased. On such vehicles, sheep, cattle and goats must after 14h of travel be given a rest period of at least 1h, sufficient for them in particular to be given liquid and, if necessary, fed. After this rest period they may be transported for a further 14h.

The Welfare of Animals at Markets Order (1990) covers animals whilst exposed for sale in a market or whilst awaiting removal after sale or when kept in a market temporarily during a journey within the UK. This requires animals to be provided with an adequate quantity of suitable food and with an adequate quantity of wholesome water before 9·00pm on the day on which the animal arrives at the market, or, if it arrives after 9·00pm, immediately on its arrival there. Thereafter, food and water should be provided at least once in each complete period of 12h (calculated from 9·00pm on the day of its arrival at the market) during which the animal is kept in the market.

1.1 Responses of sheep to transport

During transport, sheep are exposed to periods of food and water deprivation. The current legislation can be seen to allow sheep to be deprived of food for up to 29h during transport (including the 1h rest period during which food does not have to be provided), which can be followed by a further period of food deprivation at a market. The transportation process consists of a number of other factors (e.g.

assembly; loading; confinement and motion, unloading and penning), which may influence the response of sheep to the food deprivation. The number of adverse stimuli produced by the transportation and the handling involved have led to the suggestion that transport is a potential severe stressor. These stimuli have been split into a number of categories (Stephens, 1982; Connell, 1984): physical disturbances (e.g. changes in environmental factors (noxious gases, temperature etc.), agitation due to motion of the vehicle, unsuitable floor material preventing movement/standing/lying in comfort); emotional disturbances (e.g. breaking of the maternal-offspring bond, exposure to fear-provoking and/or novel stimuli, having to form and maintain new social relationships) and nutritional disturbances (e.g. food and water deprivation, changes in diet). These will act on an animal to a lesser or greater extent depending on the conditions, and the effect of a combination of these factors may be greater than when acting individually (Connell, 1984; Trunkfield & Broom, 1990).

1.1.1 Changes in liveweight

The most common effect of transport on livestock is a decrease in liveweight, partly due to excretory processes, with loss of gut fill accounting for 46·7% of total weight loss in sheep (Self & Gay, 1972; Thornton & Gracey, 1974). Hall *et al.* (1997) reported insensible weight losses in sheep (loss in weight not accounted for by loss of urine and faeces) accounted for 31-50% of the total weight loss after 14h deprivation of food and water while confined in stationary vehicles.

Although ruminants are less susceptible than non-ruminants to short periods of food and water deprivation (due to the proportionally larger size of their gut), a large proportion of the losses in both liveweight and gut contents has been observed to occur within the first 24h, with the most rapid loss in the first 12h (Smith *et al.*, 1982). Knowles *et al.* (1995) reported a linear loss of weight in sheep for the first

15h of transport, after which the rate of weight loss decreased. In addition, the large numbers of sheep going through live auctions (around 70%) results in the total transit times being greater than estimates of transport times to slaughter (Warriss *et al.*, 1990), which will contribute to increases in loss of weight.

It has been shown that provision of water during a period of food deprivation decreases the liveweight losses in cattle (Truscott & Gilbert, 1978). However, Evans *et al.* (1987) found lambs lost more weight after a 26h journey to slaughterhouse that included 5h at an auction market and 10h in a slaughterhouse lairage where they had access to water (3·07kg), than when transported direct to a slaughterhouse from a farm (5h) (0·53kg). This would suggest that the sheep did not drink sufficient water to reduce the weight loss.

Carcass weight losses occur during prolonged transportation due to dehydration of carcass tissues, mobilisation of depot fat and muscle glycogen and tissue protein catabolism (due to glucocorticoid action) (Trenkle, 1981, from Connell, 1984). In a study investigating the effect of food deprivation (3h-43h) on the carcass weight of lambs, carcass losses were found after 8h food deprivation, with losses of 0.25 kg being seen within 24h (Kirton *et al.*, 1971). Warriss *et al.* (1989) reported a reduction in carcass weight of sheep of 1·2% after a 12h food withdrawal, the loss of carcass weight increasing to 3·7% after a 72h food withdrawal.

Knowles *et al.* (1993) found that lambs transported for 14h lost significantly more weight (6·7%) than lambs held in a pen without food and water for 24h (1·5%), which suggests an effect of transport in addition to the weight loss due to withholding of food and water. This effect of transport was also found by Evans *et al.* (1987), who calculated the average rate of weight loss for each stage of a journey to a slaughterhouse. The results showed that lambs lost weight at a significantly greater rate when being transported than when in being held at an auction market or

in a slaughterhouse lairage. The increase in weight loss caused by transportation is likely to increase the carcass weight loss, increasing losses to the producer by exaggerating the effect of food and water deprivation.

Failure to feed after transport has been observed, which increases the time required for liveweights to recover to pre-transport levels. Knowles *et al.* (1995) reported that sheep fed a familiar food they would readily eat (concentrates) regained weight rapidly after 24h transport (within 24h). Sheep fed hay (an unfamiliar food) in an unfamiliar environment after 9h and 14h journeys did not readily eat the hay and took longer to regain the weight lost during transport (96h) than the lambs fed a familiar food (Knowles *et al.*, 1993). One important difference between these studies is the familiarity of the environment (and food) post-transport. In the more recent study, the sheep were returned to the pens in which they had been housed before transport, while in the earlier study sheep were transported from Scotland to the south of England. This would seem to have influenced the recovery of liveweight, mainly through reducing the intake of food.

1.1.2 Physiological response to transport

A number of studies have been published investigating the effects of transport on the physiological responses of sheep. By the nature of the treatment, they have also investigated the response of sheep to periods of food and water deprivation, as this is part of the transport process. Physiological indicators that are primarily associated with fasting will be considered later in this review. The responses discussed here are ones that are more associated with stress and dehydration and which may affect the response of sheep to food and water deprivation.

An initial increase in heart rate in sheep occurs during short-term stressful situations such as transport (Baldock & Sibly, 1990; Broom *et al.*, 1996), in response to sympathetic stimulation, and this decreases to pre-transport levels as

transportation proceeds. It has been suggested that this is due to the animals becoming familiar with the situation (Augustini & Fischer, 1982; Stephens & Rader, 1982). The increase at the start of transport has been explained in terms of physical exertion (climbing up a ramp into the vehicle, bracing to stay upright or priming muscles for escape), but changes in other physiological indicators possibly indicate an emotional as well as a physical response (Baldock & Sibly, 1990).

Transport causes an increase in plasma cortisol concentrations in sheep, the response has been found to increase to a peak within 3h of the start of the journey, decreasing during the following 3-6h (Broom *et al.*, 1996; Cockram *et al.*, 1996). It has been suggested that this increase in plasma cortisol concentration implies a stress response (Becker *et al.*, 1985; Kenny & Tarrant, 1987a, b; Fordham *et al.*, 1989). Post-transport, work in sheep has found cortisol plasma concentrations rose initially after unloading, recovering to pre-transport levels 24-48h after unloading (Knowles *et al.*, 1993). Kenny & Tarrant (1987a, b) found that the complexity of the treatment (comparing repenning cattle with confinement on a stationary vehicle and confinement on a moving vehicle) influenced both the physiological response (in terms of plasma cortisol, glucose, creatine kinase and rectal temperature) and the behavioural response of cattle, suggesting that confinement on moving trucks is the most stressful aspect of transportation for cattle.

Work has shown sheep to be at risk of dehydration during transport. Studies on sheep have demonstrated increases in osmolality, skinfold thickness and plasma total protein and albumin concentrations following 18h and 24h journeys (Knowles *et al.*, 1994b; 1996). The increased ambient temperatures experienced during journeys to France (Knowles *et al.*, 1994b; 1996) combined with the water deprivation during transportation will be the cause of the observed dehydration, as sheep housed in individual pens and deprived of food and water for 48h were found to be able to maintain normal plasma osmolality, even when exposed to an elevated

ambient temperature of 35°C (Parrott *et al.*, 1996). Shorter journeys do not appear to cause sheep to become dehydrated. Cockram *et al.* (1996) showed that sheep could be transported for 12h without experiencing any major changes in biochemical measures of dehydration or fasting.

Recovery of blood variables associated with dehydration (osmolality) to normal levels has been shown to occur by 24h post-transport. Recovery of variables associated with fasting (beta-hydroxybutyrate, urea, free fatty acids, liver glycogen) to normal levels takes longer than for variables associated with dehydration, with stabilisation occurring after 96h in lairage, but full recovery to normal levels taking up to 144h in lairage (Knowles *et al.*, 1993).

1.1.3 Behavioural response to transport

Behavioural studies have been used to complement physiological studies such as those above, thus increasing the available information on the welfare of animals being transported.

There is an increasing amount of information on the behaviour of sheep during transport. Baldock & Sibly (1990) observed that although sheep were frequently observed butting each other in a stationary trailer of sheep, the frequency decreased when the vehicle was in motion. Pearson & Kilgour (1980) also found a decrease in agonistic interactions between sheep when vehicles were motion. The high incidence of agonistic interactions between sheep during confinement is thought to be a response to the close proximity of other ewes (Baldock & Sibly, 1990). These findings agree with observations of cattle. Kenny & Tarrant (1987a) observed social interactions in cattle on stationary trucks (aggressive interactions being the most frequent), but the frequency of all social behaviours decreased as the truck began to move (particularly aggressive interactions). This phenomenon was also seen by

Eldridge (1988, from Tarrant 1990), who reported that while higher levels of social activity occurred during repenning, activity declined during trucking.

Observations of lambs during road transport have found decreases in the time spent lying down, ruminating and walking compared with pre-transport levels of these behaviours (Ewbank & Kent, 1990; Cockram *et al.*, 1996). Cockram *et al.* (1996) found that lambs that were transported had a significantly greater frequency of losses of balance, hits, rides, slips and knee-drops than those held on a vehicle at the same space allowance but not transported. A number of studies on cattle have found transport to result in a greater decrease in lying, ruminating and sleeping times than penning and starving the animals for the same period of time (Kent, 1977, from Trunkfield & Broom, 1990; Kent & Ewbank, 1983; Kenny & Tarrant, 1987a). As it has been suggested that rumination does not occur in adverse environments, only occurring when animals are settled (Beilharz, 1985), this would suggest that sheep are not settled during transport, resulting in the decreased observations in the performance of rumination.

Space allowance during the transport of sheep has been shown to have an effect on behaviour (Cockram *et al.*, 1996), with sheep at lower space allowances ($0{\cdot}22 m^2$/sheep) spending less time lying down during a 12h journey than sheep at higher space allowances ($0{\cdot}27 m^2$/sheep, $0{\cdot}31 m^2$/sheep and $0{\cdot}41\ m^2$/sheep).

Studies have shown that after 12h, 18h and 24h journeys sheep are primarily interested in feeding (Knowles *et al.*, 1994b; 1995; Cockram *et al.*, 1996), spending an increased amount of time feeding over the first few hours post-transport than under normal conditions (Cockram *et al.*, 1996). However, in two of these studies (Knowles *et al.*, 1995; Cockram *et al.*, 1996) sheep were returned to a familiar environment, which may have influenced the post-treatment behaviour.

The incidence of lying behaviour increases with time post transport in both cattle and sheep (Cockram, 1990; Kim *et al.*, 1994). The increase in lying behaviour

over time suggests the animals are acclimatising to the new environment, but the fact that cattle penned for equivalent times without being transported spend less time lying points to fatigue also being an influencing factor (Kent & Ewbank, 1983). Also, the increased feeding behaviour seen immediately after unloading increases the time before sheep begin to lie down. Knowles *et al.* (1994b; 1995) reported that sheep did not begin to lie down until after they had fed and drunk.

1.2 Feed intake and behaviour in sheep

Herbivorous species such as cattle and sheep have developed stomachs that enable the utilisation of cellulose and other plant polysaccharides that are indigestible to monogastrics (Church, 1979). The ruminant stomach has developed into a large organ (occupying nearly 3/4 of the abdominal cavity) that allows extensive microbial fermentation.

The ruminant stomach is divided into four compartments: the reticulum; the rumen; the omasum (the three pre-stomachs) and the abomasum (the true stomach). The rumen and reticulum form a fermentation vat into which food enters for about 8h. During this time, ruminal bacteria and protozoa anaerobically ferment foodstuffs, enabling the utilisation of cellulose and other crude fibres by microbial metabolism in the ruminal-reticular complex (Clarenburg, 1992). The rumen population of bacteria and protozoa is established post-nataly by inoculation and changes dynamically with alterations in diet (Currie, 1988). The microbes have a number of functions, the most important of which seem to be: digestion to transform and solubilize otherwise insoluble dietary components (e.g. cellulose); incorporation of feed materials into constituents of the microbial biomass (e.g. synthesis of amino acids and subsequent assembly of microbial protein); size reduction of fibrous food to permit passage through the reticulo-omasal orifice and into the remainder of the gut, and the production of volatile fatty acids (VFA) which are capable of providing

up to 70% of the energy requirement of the host animal (Hungate, 1966; Poppi *et al.*, 1980; Currie, 1988). The soluble products of fermentation are largely absorbed and the material leaving the rumen represents a mixture of food residues, bacteria and protozoa and some soluble fermentation products dissolved in a buffered fluid (Phillipson, 1977).

The retention time of foodstuffs would suggest that periods of food deprivation lasting longer than 8h may disrupt the supply of energy to ruminants, by reducing the production of VFA and the amount of material available for absorption.

1.2.1 Feeding behaviour

Sheep have a mobile upper lip that permits very close grazing and allows them to be selective when grazing. Sheep do not graze continuously, but in cycles interrupted by periods of rumination, rest and idling. Sheep at pasture spend 9-11h/day grazing in 4-7 grazing periods (Tribe 1949a; Schneider *et al.*, 1953) compared with cattle who graze for 4-9h/day in 4-5 grazing periods (Hafez *et al.*, 1969). In general, intensive grazing begins around sun-rise and stops about sun-down. The longest periods of grazing occur in the early morning and between late afternoon and dusk (Hughes & Reid, 1951), but temperature, day length and fly prevalence will influence the incidence of night grazing, with increased night grazing (between 19·00h and 07·00h) during summer months (June-September) (Tribe, 1949b). The feeding bouts last for 20-90 minutes and are followed by a period of 45-90 minutes during which the sheep will ruminate or rest (Lynch *et al.*, 1992).

Studies indicate that sheep will spend 8-9h/day ruminating (Pearce & Moir, 1964; Pearce, 1965; Weston & Hogan, 1967; Welch & Smith, 1968, 1969). Rumination involves the regurgitation of ingesta from the reticulo-rumen through the aspiration of reticular contents into the oesophagus and anti-peristaltic waves of contractions moving the digesta into the mouth (Stevens & Sellers, 1960; Winship *et*

al., 1964; Dougherty *et al.*, 1971). Rumination breaks food down sufficiently to leave the rumen. After exposure to a rumen environment for 10 days without rumination, 2cm lengths of hay were not reduced to a size that would permit them to pass through the reticulo-omasal orifice (Welch, 1982). A relationship between the amount of rumination and the fibre content of the diet has been established, with a correlation between cell wall constituent intake and ruminant activity (Welch & Smith, 1969, 1970; van Soest & Wine, 1967). Feeding grain or finely ground roughage causes a cessation of rumination or pseudo-rumination (Balch, 1952, 1971; Gordon, 1958; Freer & Campling, 1965; Welch & Smith, 1970, 1971). The lower amount of rumination seen during transport may increase the retention time of food during transport, which may affect the response of the sheep to the period of food deprivation.

1.2.2 Response to food deprivation

A number of indicators that respond to fasting in sheep have been identified. Warriss *et al.* (1989) observed that increases in beta-hydroxybutyrate concentrations were indicative of the length of time sheep had been without food. Plasma concentrations increased progressively in samples taken from sheep after food deprivations of 0h, 12h, 24h, 36h, 48h, or 72h. Beta-hydroxybutyrate is formed from the VFA acetate in the absence of sufficient oxaloacetic acid (i.e. inadequate propionate). The lack of sufficient propionate also affects the blood glucose concentrations, as this is a major source of glucose for the ruminant. In fasted sheep, blood glucose concentration is reduced (Lindsay & Leat, 1975), decreases in the blood glucose concentration of sheep have been reported after 24h fasting (Warriss *et. al.*, 1989; Knowles *et al.*, 1995). There is also an increase in free fatty acid concentration due to the catabolism of body fat reserves to meet the energy requirement of the sheep (Warriss *et al.*, 1987). In sheep, a 12h food deprivation has

been found to increase the plasma free fatty acid concentration, with increased plasma beta-hydroxybutyrate concentration found after 24h food deprivation (Cockram *et al.*, 1997). Both these indicators were found to decrease during the first 3h of access to food and water (Cockram *et al.*, 1997).

After periods of food deprivation animals have been observed to consume more feed than under *ad-libitum* conditions (Levitsky *et al.*, 1976). This has been seen in sheep after 12h (Cockram *et al.*, 1996; Sibbald, 1997), 14h (Hall *et al.*, 1997), 18h (Sibbald, 1997) and 24h food deprivation (Cockram, *et al.*, 1997), with increased eating and decreased lying seen during the first 12h post-deprivation compared with the equivalent time pre-deprivation (Cockram *et al.*, 1996). Hall *et al.* (1997) found that after 14h deprivation sheep consumed a large amount of food during the first hour (especially if offered familiar concentrates rather than hay), consuming smaller amounts over the next 3h. After 12h and 18h food deprivation, Sibbald (1997) found that hourly intakes of dried grass pellets increased, especially during the first hour post-deprivation. After fasting for 16h and 24h, Dumont & Petit (1995) observed that sheep were willing to consume more of a low quality forage that was readily available, rather than work for greater access to restricted amounts of high quality forage. In this study, sheep were required to walk around a race at each end of which a large quantity of low quality forage and a small amount of high quality forage was available.

In all these studies, apart from Hall *et al.* (1997), sheep were held in or returned to a familiar pen, and fed familiar food following the period of deprivation. This does not occur during the commercial transportation of animals. Knowles *et al.* (1993) reported that sheep seemed unwilling to consume an unfamiliar food following 9h and 14h journeys from Scotland to the south of England. This would suggest that the change in environment (location, diet etc.) may affect the response of sheep to a period of food deprivation in terms of behaviour when they are given

access to food and water following the period of deprivation, and the recovery of physiological indicators to normal levels..

1.2.3 Control of food intake

A range of possible mechanisms controlling food intake have been examined, with a large amount of work looking at the effects of single factors on food intake. The main conclusions from over 50 years of work in this area are reviewed here.

Feedback mechanisms

Feedback mechanisms potentially provide the animal with a method of terminating food intake before absorption of nutrients from the meal has taken place. Receptors have been identified throughout the gastro-intestinal tract providing sensory perception of the food in the mouth (through receptors in the buccal cavity and throat, Zeigler, 1975), in the stomach (Janowitz & Hollander, 1955; Balch & Campling, 1962) and of digesta reaching the duodenum and jejunum (Canbeyli & Koopmans, 1984; Houpt, 1985). Work has shown that physical distension of the rumen influences food intake. Removal of swallowed food caused cattle to consume more food than usual, and insertion of food or bladders containing water have been shown to depress intake in cattle (Schalk & Amadon, 1928, from Balch & Campling, 1962; Campling & Balch, 1961). This would seem to be an important factor in the control of food intake, as it provides a rapid feedback mechanism as the animal is feeding. Infusion of a liquid diet into the jejunum of rats was found to reduce food intake (Canbeyli & Koopmans, 1984), as was the physical distension of the small intestine in pigs (Houpt, 1985).

In addition, since the early 1970's, attention has turned to the liver as a potential factor involved in feedback control, due to the good blood supply, and the important links to the CNS (to the ventromedial nucleus of the hypothalamus and the

lateral hypothalamic area) (Anil & Forbes, 1987). A range of factors have been demonstrated to affect intake through the liver. For example, infusion of glucose into the portal vein depressed food intake in dogs (Russek, 1963), and infusion of 2-deoxy-D-glucose (a glucose antimetabolite which blocks the uptake of glucose by the liver) into the hepatic portal vein stimulated food intake in rabbits (Novin *et al.*, 1974). Work on sheep has shown effects of the liver in conjunction with propionate on food intake (Anil & Forbes, 1980). Infusions of this fatty acid into the hepatic portal and jugular vein cause a dose-dependent depression of food intake, with a greater reduction following infusion into the portal vein. Sheep that had their liver denervated did not respond in the same way to infusion of propionate, 95% denervation of the liver resulted in sheep eating normally after infusion of the fatty acid (Anil & Forbes, 1980). These results suggest that the liver is the major site mediating the effect of propionate on food intake. The results also demonstrate the interactions that occur between factors that have been put forward separately as potentially important regulators of food intake.

Gastrointestinal tract

Rumen capacity has been investigated as a physical restrictor of food intake (Balch & Campling, 1962). Putting a balloon in the rumen and filling it with water (thereby reducing the available space) has been shown to decrease food intake in cattle (Campling & Balch, 1961; Anil et al., 1993) and sheep (Davies, 1962). Studies on sheep with similar balloons placed in the rumen, found that when fed pelleted diets, there was some evidence of a recovery in food intake towards pre-treatment levels, that was not seen when sheep were fed straw (Egan, 1972). The diet would seem to have been important in this recovery in food intake.

Infusion of volatile fatty acids into the rumen have been observed to decrease food intake (Baile & Forbes, 1974; de Jong, 1986) with a significant relationship

between the amount of VFA infused and the extent of the depression in food intake (Baile & Mayer, 1969). Studies have shown the decrease in food intake to be mainly due to acetate and propionate, acting via receptors on the rumen wall (acetate and propionate) and in the abomasum and duodenum (propionate) (Baile, 1971; Baile & McLaughlin, 1970).

Hormonal control

The effects of a range of hormones have been investigated. Injections of insulin has been found to stimulate feeding in sheep (Baile *et al.*, 1969; Seoane *et al.*, 1984). Other hormones have been found to depress food intake in sheep (e.g. cholecystokinin (CCK): Della-Fera & Baile, 1979) and in rodents (e.g. glucagon, somatostatin, and pancreatic polypeptides: Baile & Martin, 1971; Lotter *et al.*, 1981; McLaughlin & Baile, 1981; Geary & Smith, 1982). Morley & Levine (1980) suggested that whilst hormones have been shown to affect intake in a range of species, they do not play a central role in the control of food intake. However, Crawley *et al.* (1984) have suggested a mechanism by which such peripheral signals (in this case CCK) could be integrated with CNS signals in the control of food intake. This involves a sensory feedback pathway from the gastrointestinal regions (gut CCK receptors), via the vagus nerve through the nucleus tractus solitarius (NTS) to the forebrain regions mediating feeding. Rats with lesions that completely destroyed all ascending nucleus tractus solitarius projections, did not show the reduction in feeding and exploratory behaviour following administration of CCK, that has been demonstrated in intact animals, providing evidence for such a mechanism, as the ascending NTS fibres were necessary for the CCK response to be seen (Crawley *et al.*, 1984). A potential problem with such work is the extent of the damage caused when such lesions are made, as other fibres that may also be involved with the control of food intake, or some other pathway may also be damaged.

However, in the case of Crawley *et al.* (1984) partial and incorrect lesions (which still damaged other fibres) did not abolish the CCK effects, suggesting that any other fibres damaged by the procedure had little effect on the pathway being investigated.

Central nervous system

Various areas of the brain have been associated with feed intake. Stimulation of the hypothalamus has been shown to stimulate feeding, and destruction of this area results in aphagia (Baile *et al.*, 1968). The ventromedial area (Baile *et al.*, 1969), paraventicular nucleus and rostral areas such as the nucleus of the solitary tract and the area postrema (Crawley *et al.*, 1984; Willis *et al.*, 1984) have also been shown to be involved with food intake. Workers have demonstrated the existence of pathways connecting these areas (Crawley *et al.*, 1984; Palkovits *et al.*, 1984). Effects of neurotransmitters and neuropeptides on food intake have also been demonstrated, with the administration of neuropeptides and neurotransmitter agonists stimulating feeding in a range of species (rats: Wise & Dawson, 1974; sheep: Baile & McLaughlin, 1987; Della-Fera *et al.*, 1990; pigs: Baldwin *et al.*, 1990).

Recent reviews have concluded that theories resulting from work on single factor control of food intake do not provide explanations for control of food intake under all possible circumstances (Balch & Campling, 1962; Baile & McLaughlin, 1987; Forbes, 1996). A number of theories have been put forward which integrate a number of factors to control the food intake. Forbes (1995) describes a number of these, including theories using energy supply to tissues (Le Magnen, 1976; Le Magnen & Devos, 1984); 'hedonic' properties of food (Armitage *et al.*, 1983) and multiple feedbacks (Anil & Forbes, 1980) to control food intake. Theories involving energy supply use the concept that the energy supply to some tissues is monitored and used to control intake. Studies investigating the meal-to-meal energy balance

(difference between the caloric intake of a meal and the energy expenditure from the start of one meal until the start of the next meal) and medium term energy balance (6 to 24h) in rats demonstrated an excess of energy intake at night and a deficit of energy intake during the daytime. This excess of intake at night has been shown to be correlated with the deficit of the day, resulting in a 24h balance that is close to equilibrium (Le Magnen & Devos, 1982; 1984; Le Magnen, 1985). If this theory of the control of food intake also applies to ruminants, it will have consequences for periods of food deprivation which last beyond one day or one night, as the lack of intake during one part of the day would be compensated for during the next part of the day. This would suggest that a gradual increase in the motivation to obtain food occurs, as the length of food deprivation extends.

Hedonic properties of food affect the acceptability of a food and enable animals to avoid a particular food if it has negative consequences. Offering rats highly palatable foods in addition to a standard rat diet that was supplied *ad-libitum*, resulted in an increased energy intake, with an increase in the energy expenditure of the rats, which was not sufficient to prevent weight gain (Armitage *et al.*, 1983). This weight gain would suggest that the palatability of the foods offered overrides the ability of the rat to maintain energy balance over a 24h period. However, Armitage *et al.* (1983) observed a falling off of food (and hence) energy intake over the final 2 weeks of the trial. This theory would suggest that the control of energy balance was adjusting to the new energy-rich diet that was available, and with time, the rats may have attained a new steady state. This would seem to involve the long-term regulation of food intake, and so will have little effect on the response of sheep to relatively short periods of food deprivation (such as those associated with transport).

Multiple feedback theory is based upon the fact that in trials investigating single factors involved in the control of food intake, the treatments are applied at

levels far in excess of the biological levels found in the animals, and do not explain how physiological changes work to produce the same results. Examples in sheep include the infusion of propionate into the hepatic portal vein, acetate into the rumen, and rumen distension which resulted in an additive effect on reducing the intake of food (Adams & Forbes, 1981; Forbes, 1996). This type of control of food intake allows for additivity between a number of single factors, and the integration of a number of stimuli (like those previously discussed), resulting in the stimulation of a wide range of receptors influencing the response of other sites, perhaps via the central nervous system (Forbes, 1996). Because of the encompassing nature of this theory, it would seem to be the most appropriate for the control of food intake, as it also incorporates the theories already discussed. This results in a theory that can cope with both short term and long term control of feed intake, by accepting the involvement of a whole range of factors.

1.3 Measuring feeding motivation

Objective methods of measuring motivation are important, as they may provide information as to the way animals experience subjective feelings (Petherick & Rutter, 1990). Lawrence *et al.* (1993) described motivations as 'temporary and reversible brain states induced by internal and external signals resulting in the tendency to perform specific behaviours'. For any motivational system, the behavioural tendency arising from the combination of internal and external factors 'competes' with other tendencies to be expressed, and the expression will alter the balance between other tendencies as to which are expressed and which are inhibited. A classic example of this is the time-sharing between eating and drinking observed in Barbary doves that have been deprived of food and water. When the birds are given access to food and water they are observed to switch between the behaviours until satiated (Sibly, 1975; Sibly & McFarland, 1976).

Keeping animals free from hunger and thirst is contained in most codes of recommendations for the welfare of animals (e.g. Fraser & Broom, 1990; FAWC, 1992). Hunger has been described as the state of the animal in which it is stimulated to eat (Le Magnen, 1985), but there has been criticism of this as a simplistic definition as it does not take account of the 'nutritional properties of the food, the physiological state of the animal or the external factors that could influence/prevent feeding' (Kyriazakis & Savory, 1997). These criticisms apply more to the use of hunger as an alternative term for feeding motivation. Such a definition also does not include the concept of hunger as an emotional state/feeling of an animal that it can perceive and could suffer from if the hunger was prolonged. An explanation of motivational systems has developed from the approach of McFarland and others using a 'state-space' approach (McFarland & Houston, 1981), with the motivational state represented as a point in a multi-dimensional space, with the axes representing internal and external causal factors that elicit the state. This places 'hunger' as an internal state that acts in combination with external stimuli (such as the properties of the food) resulting in the feeding motivation. This seems a more satisfactory use of the term 'hunger' as it allows for differences in feeding behaviour that can be observed in animals with access to different food types; whilst the internal hunger state may be similar, the reaction to the provided food may vary depending on the food provided, or the location it is provided in. For example, Burritt & Provenza (1997) have found that sheep are unwilling to consume novel foods in unfamiliar environments, and when in a familiar environment, food restriction does not increase the intake of a novel food (Provenza *et al.*, 1995). The Le Magnen definition of hunger would suggest that the food restricted sheep offered a novel food are not hungry, as they are not stimulated to eat the food provided. The McFarland & Houston use of the term 'hunger' allows the effect of the novel food or the novel environment to be taken in to account in explaining the change in motivation to feed.

A number of techniques have been developed to attempt to measure feeding motivation. They tend to fall into three categories: the direct measurement of food intake, rate of eating and the time spent in food-directed activities; methods that require the animals to work in order to obtain food (e.g. operant conditioning), and aversive stimuli that animals must undergo to obtain food (e.g. electric shocks, water baths).

1.3.1 Direct measurement

Miller *et al.* (1950) demonstrated limitations with using total food consumption as a measure of 'hunger'. Rats with hypothalamic lesions that have been shown to result in obesity were found to eat more food than controls when offered *ad-libitum* access. However, when the rats were required to work for food (e.g. bar pressing, running down an alley) or overcome resistance (e.g. tolerate quinine, or electric shocks), they were found to work less hard and be more easily deterred from eating. Miller (1955) compared the use of food intake, operant response and aversive stimuli after periods of food deprivation. Food intake was found to reach a maximum after 30h food deprivation, whilst the other methods continued to increase with length of food deprivation. It would seem that the method to be used may depend on the length of deprivation being investigated.

An alternative to food intake is measuring the rate of intake when food is first provided. This has been shown to be correlated with length of food deprivation in poultry (Wood-Gush & Gower, 1968; Savory, 1988).

A number of studies have used direct measurement of intake to investigate the effects of food restriction and deprivation on the responses of sheep. Hall *et al.* (1997) investigated the food and water intake of sheep following a 14h food and water deprivation. The intakes of hay or concentrates by sheep that had previous experience of both these foods, and by sheep with no experience of them, were

measured over the first 7h post-deprivation. Previous experience of the food offered was found to have an effect on post-deprivation intake. Sheep familiar with the food consumed a large quantity of concentrates during the first hour post-treatment (>1kg/sheep), and a lower quantity of hay (<0·5kg/sheep). Sheep unfamiliar with both types of food consumed similar amounts of both foods (<0·1kg) but significantly less than sheep that were familiar with the food offered. This imposes restrictions on the use of this technique to investigate the feeding motivation of sheep, as the results can be seen to be influenced by the diet offered to the sheep, in terms of whether they are familiar with the food being offered and whether they have a preference for it.

1.3.2 Working for food

Operant conditioning involves the repeated emission of the same response for a reward (Honig, 1966) and provides a method of studying the feeding motivation of animals in terms of the work they will do for a small reward of food.

Operant experiments with sheep as the experimental animal have been performed, although to a lesser extent than other species such as pigs and chickens (Kilgour *et al.*, 1991). The responses used in all species have varied. In sheep responses have included breaking an infra-red beam with their noses (Baldwin, 1972; 1975; Baldwin & Start, 1978, 1981) and panel pressing (Baldwin, 1981; Blisset *et. al.*, 1990). Most of the operant conditioning work with sheep has used food as the reward for performing the required response, but operant conditioning has not been used to investigate the feeding motivation of sheep. Baldwin (1981) used this method to assess shape discrimination. Operant conditioning has also been used for olfactory discrimination of conspecifics (Baldwin & Meese, 1977), and for distinguishing oestrus/non-oestrus ewes by rams (Blisset *et al.*, 1990). Panel

pressing has also been used to look at food preferences of sheep (Hutson & van Mourik, 1981), with the highest rates of response for barley and wheat.

Operant conditioning has been used to investigate the feeding motivation of pigs on restricted diets in terms of how much work the pigs would do for access to limited amounts of food (6g of pellets), when fed diets ranging from 0·8 to 0·4 of their predicted *ad libitum* food intake, or from 3·5 times maintenance level to 1·5 times maintenance level (e.g. Lawrence *et al.*, 1988; 1989; Lawrence & Illius, 1989). Such work has found that operant rates increased with the level of food restriction, leading the authors to suggest that this indicated an increased feeding tendency (Lawrence *et al.*, 1993).

The use of push-doors is an alternative to the use of operant conditioning techniques in that it also measures how hard animals will work for access to the reward by using physical force rather than repetition of a behaviour. Operant conditioning techniques have a number of problems including constraints on learning (Shettleworth, 1972), and the high cost of the equipment. The use of push-doors is a cheaper alternative that has been used to measure a number of different motivations in poultry (Duncan & Kite, 1987; Petherick & Rutter, 1990).

Dumont & Petit (1995) trained sheep to walk around an arena to obtain food. At each end of the arena limited quantities of high quality forage (<10g) and *ad-libitum* low quality forage were available. Depriving the sheep of food demonstrated a change in the dietary preferences of sheep, with increased intake of the low quality forage but no change in the intake of the high quality forage. This may indicate an increased motivation to feed, both by the increased intake and the increased willingness to eat the low quality forage.

1.3.3 Aversive stimuli

In a review of aversion, (Rushen, 1986b) the range of stimuli that can be used in aversion studies were categorised into three different procedures: punishment, avoidance learning and aversive classical conditioning. Aversive classical conditioning involves the pairing of the aversive stimulus with a signal indicating the onset of the stimulus. For example, signalling the onset of an electric shock with a cue such as a light or buzzer. Punishment involves exposing the animal to the aversive stimulus immediately after it has performed the required behaviour. Examples of the use of this technique include that of pigs that were trained to panel push for food and given an electric shock when a food reward was obtained (Dantzer & Baldwin, 1974), and the assessment of the relative aversiveness of a range of handling procedures to sheep in terms of the effect the handling procedure (physical restraint or electro-immobilization) had on the time taken to run a race (Rushen, 1986a). This method has not been found to be effective in assessing feeding motivation, as periods of food deprivation reduced the effectiveness of punishment in suppressing the behaviours learnt to obtain food (Miller, 1959; Azrin, 1960; Azrin *et al.*, 1963).

Petherick *et al.* (1992) exposed chickens to an aversive stimulus after periods of food deprivation. The chickens were required to move along a race to a goal box containing food. When the chickens reached the food, they were exposed to the aversive stimulus so that it was associated with running along the alley and entering the goal box. Miller (1955) reported studies using food adulterated with quinine, and found that rats that had been food deprived tolerated higher concentrations of quinine.

Avoidance learning involves exposing an animal to an aversive stimulus if it does not perform a 'designated' behaviour. This should result in an increase in the occurrence of the behaviour. This has been used with poultry, where hens were

exposed to an inflating balloon (previously shown to be an aversive stimulus to poultry (Duncan & Filshie, 1979) if they did not move into a compartment when a light was switched on (Rutter & Duncan, 1991). This technique was not readily learnt by the hens being tested, and has mainly been used with behaviours other than feeding, for example, Bailey *et al.* (1983) used this technique to assess the aversiveness of the vibration associated with transport in pigs. The pigs were trained to press a panel to stop the vibration of the pen in which it was held, or the noise of the vibrator. They compared the rate at whcih pigs would perform the response and found that pigs would use the panel to stop the vibration, but not to stop the noise. This gave some indication of the aversiveness of these two components of transport.

1.4 Conclusions

The control of food intake and the factors controlling feeding motivation in ruminants are not yet fully understood. Work that has been conducted would suggest that following periods of food deprivation, an increased motivation to feed exists in sheep. This is demonstrated by changes in blood metabolites associated with fasting and increases in the amount of feeding behaviour observed when food is returned to the sheep, following a period of food deprivation. However, it is not yet clear how long such an increased interest in feeding will last, or how such a response may be affected by other variables associated with transport (provision of food and water during transport, changes in diet etc.). It is also not yet known how strong this interest in food is after a period of food deprivation and whether it is more than just a response to the changes in the plasma concentrations of metabolites which have been shown to be affected by periods of food deprivation. A number of methods have been developed to measure the motivation of animals to perform various behaviours, and while there is still a limited volume of work on feeding motivation in sheep,

such methods have been developed with other species and it should be possible to adapt these methods to be used with sheep.

The programme of work undertaken in this thesis set out to identify the behavioural responses of sheep to periods of food deprivation that can be experienced during transport. It also investigated a number of factors associated with transport that may influence the feeding behaviour of sheep post-transport. In addition, the feeding motivation of sheep post-deprivation was investigated using some of the methods that have been used in other species.

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CHAPTER 2 Effect of food deprivations of up to 24h on the behaviour of sheep

Abstract

An experiment was designed to investigate the effect of food deprivations of up to 24h on the behaviour of sheep. Behavioural observations were made on pairs of adult sheep (n=7) before, during and after food deprivations of 6h, 12h, 18h and 24h. During periods of food deprivation (treatment) sheep spent a greater proportion of scans foraging (0·14) and standing (0·53) and a lower proportion of scans ruminating (0·17) and lying (0·45) than when they had access to food (foraging: 0·03; standing: 0·41; ruminating: 0·32; lying: 0·57). Sheep also took a greater number of steps during periods of food deprivation (764) than when they had access to food (738). Sheep drank less water during the periods of food deprivation (0·8l) than when they had access to food (10·1l).

Sheep ate more hay post-treatment (5·9kg) and spent a greater proportion of scans eating (0·65) during the first 5h post-treatment than during the equivalent time pre-treatment (4·6kg, 0·28). This increase in feeding was seen after each period of food deprivation. During the first 6h post-treatment, sheep took a greater number of steps (331) than during the equivalent 6h pre-treatment (226).

An increase in foraging and a greater number of steps taken suggests that sheep begin to actively seek food during food deprivation. The increase in feeding post-treatment was similar for each length of food deprivation, suggesting the effect of 6h-24h food deprivation on feeding behaviour was independent of time since the last meal.

2.1 Introduction

Sheep can be exposed to short-term periods of food deprivation during transportation, at markets and at slaughterhouse lairages. Whilst ruminants are less susceptible than non-ruminants to short periods of food and water deprivation due to the proportionally larger size of their gut, little is currently known of the psychological effects of short-term food deprivation on sheep.

Many studies involving fasting sheep for relatively short periods have been performed to investigate energy metabolism (Blaxter, 1962; 1967; Corbett *et al.*, 1980; Thomson *et al.*, 1980). These have generally involved housing sheep individually in metabolism crates and calorimeters which may restrict behavioural expression (Bowers *et al.*, 1993). Other studies involving similar lengths of food deprivation have investigated the effects of transportation on the behaviour and physiology of sheep (Knowles *et al.*, 1993; 1994; 1995; 1996; Cockram *et al.*, 1996).

Such studies involve a number of additional factors, including the moving of sheep to novel environments, motion of the vehicles and close confinement with unfamiliar sheep during the period of deprivation (Knowles *et al.*, 1993; 1994; 1995; 1996; Cockram *et al.*, 1996). Sheep spend an increased amount of time feeding after 12h journeys (Cockram *et al.*, 1996). After longer journeys of 18h and 24h to mainland Europe, changes in blood metabolites indicative of the mobilisation of body reserves have been found in sheep (Knowles *et al.*, 1994; 1995). The changes in blood metabolites in association with an increased interest in feed post-transport may indicate that the sheep are experiencing a form of 'hunger' or an increase in feeding motivation.

A number of authors have suggested that behavioural methods are more sensitive measures of welfare than physiological responses, (Syme & Elphick, 1982; Mellor & Murray, 1989; Molony, 1991). Duncan (1974) felt that behavioural responses could be quicker and more subtle at indicating when the welfare of an animal had been compromised The behavioural response of an animal will alter depending on whether the conditions to which they are exposed are short/long term, with short term potential stressors (such as short term food deprivation) producing diverse behavioural responses that alter according to the stimuli (Broom, 1988).

This experiment was performed to investigate the effect of removing food for up to 24h on sheep kept in familiar surroundings at high space allowances without exposure to additional stressors. In particular, it examined the behaviour of sheep during a period of food deprivation and the effect of the length of the deprivation on the post-deprivation behaviour.

2.2 Material and methods

Fourteen ewes (Suffolk and Suffolk crosses) aged between 1 and 3 years were used. The sheep were randomly allocated to pairs and penned on wood-shavings (2·0m x 2·0m). All sheep were individually marked with a stock marker (Super Sprayline Stockmarker, Ritchey Tagg Ltd.) to enable identification of each sheep during the behavioural observations. The sheep were offered known amounts of hay (3-4 kg) and water (9 litres) twice a day (at 07.30h and 14.00h).

2.2.1 Protocol

The sheep were exposed to four periods of food deprivation: 6h, 12h, 18h and 24h, during which they had continuous access to water. The treatments were carried out in a random order over four consecutive weeks. A 6-day recovery period was allowed between treatments to conform with the time taken by sheep to recover physiologically from 24h of transport (Knowles *et al.*, 1993).

The behaviour of the sheep was recorded using video cameras located outside the pens, connected to time lapse video recorders. The behaviour was recorded for 24h (pre-treatment), when they had access to hay and water (07.30h-07.30h). At 07.30h the next morning, the hay was removed from the pen, fresh water was added and the sheep were videoed for the duration of the food deprivation (treatment). On completion of the treatment, known amounts of hay and water were provided, and the sheep were videoed for a further 24h (post-treatment). Food and water intake for each pen were measured by weighing and measuring refusals at 07.30h and 14.00h.

2.2.2 Behavioural observations

The video tapes were analysed by scan sampling each sheep every 5 minutes. The behaviours recorded were:

standing (upright stationary posture);

lying (recumbent posture with body in contact with floor);

walking (upright posture involving a change in location);

eating (chewing and swallowing of food);

ruminating (regurgitation, chewing and reswallowing a bolus of food);

foraging (nosing through bedding-may or may not be followed by eating)

other (any other behaviour not described above).

Continuous observations of the number of steps taken and the incidence of drinking (muzzle immersed in water for >5s) and butting (striking another sheep with head) were recorded for each sheep.

2.2.3 Statistical analysis

For each sheep, the proportion of scans spent in each behaviour was calculated for each hour of the observation periods. From these values, the mean proportion of scans spent in each behaviour were calculated for each pen (i.e. each pair of sheep). These means were used for statistical analysis, giving n=7. A repeated measures analysis of variance (Laird & Ware, 1982) using the mixed procedure within SAS version 6 (SAS Institute Inc., Cary USA) was used to examine the effects of treatment (deprivation length) and time on the mean proportion of scans spent performing each behaviour. Where there were interactions between treatment and time, the differences between the least-square means were examined. Analyses were performed to make specific hourly comparisons during equivalent times of day for: the four treatment periods and the equivalent times pre-treatment; the first 12h post-treatment and the equivalent time pre-treatment and the second 12h pre-treatment and the equivalent time pre-treatment.

This analysis was repeated to examine the effects of treatment (deprivation length) and time on the hay and water intakes, comparing the intake of water during

treatment with the pre-treatment intake, and the post-treatment intakes of hay and water with the pre-treatment intakes.

The relationship between the post-treatment performance of each recorded behaviour and the length of the food deprivation was investigated by using linear regression analysis. If data were skewed, the regression was conducted on transformed data (log10). The linear regression lines and their residual sum of squares were calculated for each behaviour regressed against the length of food deprivation (Ryan *et al.*, 1992).

For each sheep, the number of steps taken were totalled over 6h periods. From these values, the mean number of steps taken over each 6h period were calculated for each pen. This was also done for the incidence of butts and drinking bouts. A Friedman two-way analysis of variance was used to examine the effect of deprivation length (treatment) on these means. Where significant effects of deprivation length were found, multiple comparisons were used to determine which treatments differed significantly. This was done by calculating the differences between the sum of the ranks for each pair of treatments and comparing this with the critical difference (Siegel & Castellan, 1988). The frequency of these behaviours during the treatment periods and the post-treatment periods were compared with the frequency during the pre-treatment periods using the Wilcoxon signed ranks test.

2.3 Results

2.3.1 Hay and water intakes

Table I shows the mean hay and water intakes during the 24h pre-treatment period, the treatment period and the 24h post-treatment period.

Table I. Mean hay and water intakes (s.e.) before, during and after food deprivation (n=7).

	Pre-treatment	During treatment	Post-treatment
Hay Intake (kg)	4·6[a]	-	5·9[b]
	(0·15)	-	(0·19)
Water Intake (litres)	10·1[a]	0·8[b]	10·6[a]
	(0·52)	(0·22)	(0·50)

a, b. Values with different superscripts in the same row differ significantly ($p<0·0001$).

There was no effect of the length of food deprivation on either the hay or water intake post-treatment or on the water intake during treatment. Sheep ate significantly more hay post-treatment than pre-treatment (Table I, $p<0{\cdot}0001$), but there was no change in water intake post-treatment. Sheep drank less water during the treatment periods than during the pre- and post-treatment periods (Table I, $p<0{\cdot}0001$). There was no effect of deprivation length on the mean volume of water drunk during treatment (means: 6h: 0·2 litres, s.e. 0·07; 12h: 0·6 litres, s.e. 0·31; 18h: 1·1 litres, s.e. 0·43; 24h: 1·3 litres, s.e. 0·76).

During the first 12h of treatment, sheep had a lower number of drinking bouts than at the equivalent time pre-treatment (median (Q1, Q3): pre-treatment: 7 (6, 9); during treatment: 1 (0, 2), $p<0{\cdot}0001$). During the first 6h post-treatment, the number of drinking bouts was greater than during the equivalent time pre-treatment (median (Q1, Q3): pre-treatment: 1 (0, 4); post-treatment: 4 (3, 5), $p<0{\cdot}01$). There was an effect of deprivation length over the whole 24h post-treatment period, with the greatest number of drinking bouts after 24h food deprivation and the least after 18h food deprivation (median (Q1, Q3): 6h: 8 (7, 8);12h: 8 (7, 9); 18h: 7 (5, 9); 24h: 10 (9, 10), $p<0{\cdot}05$). The length of food deprivation was found to be a good predictor of the incidence of drinking during the second 12h post-treatment, with the incidence of drinking decreasing as the length of food deprivation increased (Table IV, $p<0{\cdot}01$).

2.3.2 Behaviour

During treatment

There was a significant effect of time and the time*deprivation length interaction on the proportion of scans spent performing all behaviours except walking. Table II shows the mean percentages spent in each behaviour during treatment and during the equivalent time pre-treatment for the four deprivation lengths.

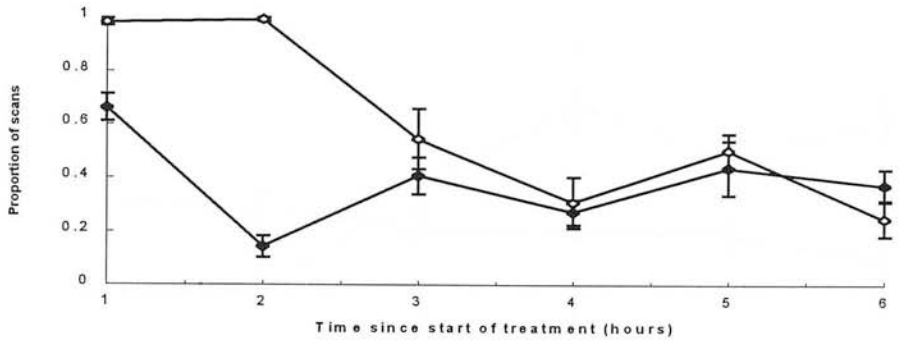
Figure 2.1 shows the mean proportion of scans spent standing during the period of food deprivation and the equivalent time pre-treatment for the four lengths of food deprivation. During the first 4h of treatment a lower proportion of scans were spent standing ($p<0{\cdot}0001$), and a greater proportion of scans were spent lying

($p<0{\cdot}0001$) than at the equivalent time pre-treatment. This increase in the proportion of scans spent lying and decrease in the proportion of scans spent standing was seen during each length of food deprivation, during the first 2h for 6h food deprivation (Figure 2.1a), and during the first 3h for 12h, 18h and 24h food deprivation (Figure 2.1b-d).

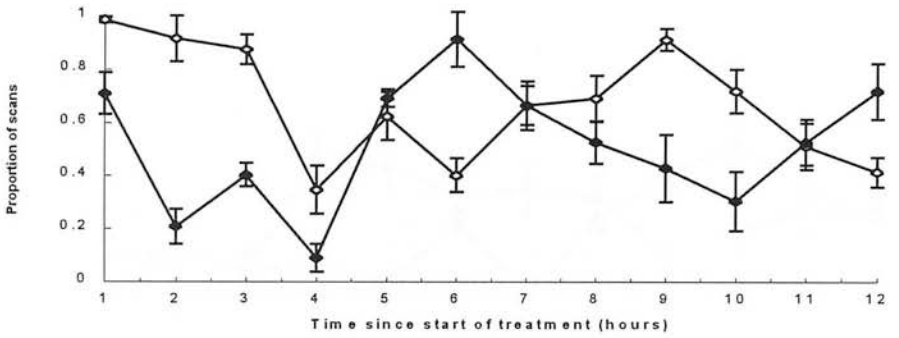
Table II. Mean percentage of scans (s.e.) spent performing each behaviour during food deprivation and during the equivalent time pre-treatment (n=7).

Length of deprivation(h)	Pre-treatment	During treatment
Lying		
6	0·37(0·07)	0·61(0·07)
12	0·31(0·11)	0·38(0·11)
18	0·54(0·03)	0·60(0·02)
24	0·60(0·09)	0·67(0·08)
Standing		
6	0·60(0·07)	0·38(0·06)
12	0·67(0·14)	0·52(0·13)
18	0·45(0·07)	0·40(0·07)
24	0·39(0·08)	0·32(0·07)
Walking		
6	0·01(0·004)	0.002(0.001)
12	0·01(0·002)	0·01(0.005)
18	0·01(0·01)	0·004(0.003)
24	0.01(0·01)	0·01(0.008)
Foraging		
6	0·04(0·04)	0·14(0·04)
12	0·02(0·03)	0·07(0·03)
18	0·02(0·03)	0·19(0·03)
24	0·02(0·04)	0·16(0·03)
Ruminating		
6	0·37(0·04)	0·24(0·02)
12	0·28(0·03)	0·23(0·01)
18	0·30(0·06)	0·10(0·03)
24	0·33(0·06)	0·12(0·06)
Other		
6	0·01(0.003)	0.001(0.004)
12	0.01(0·01)	0·02(0.01)
18	0.01(0.01)	0.004(0.006)
24	0.01(0.006)	0.002(0.004)

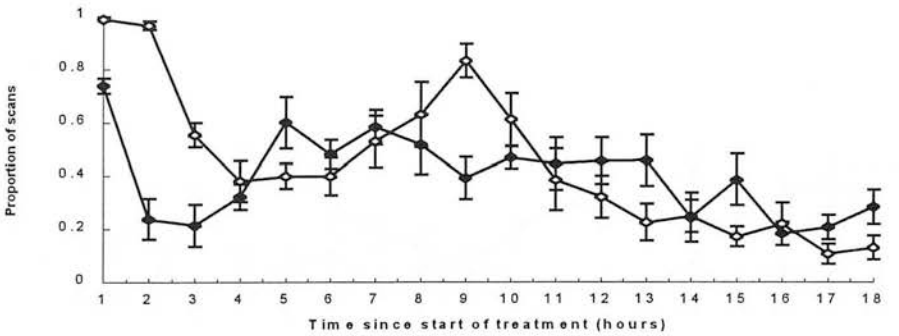
After the first 12h of treatment, few differences were seen in the proportion of scans spent lying and standing during 18h and 24h food deprivations compared with the equivalent time pre-treatment.



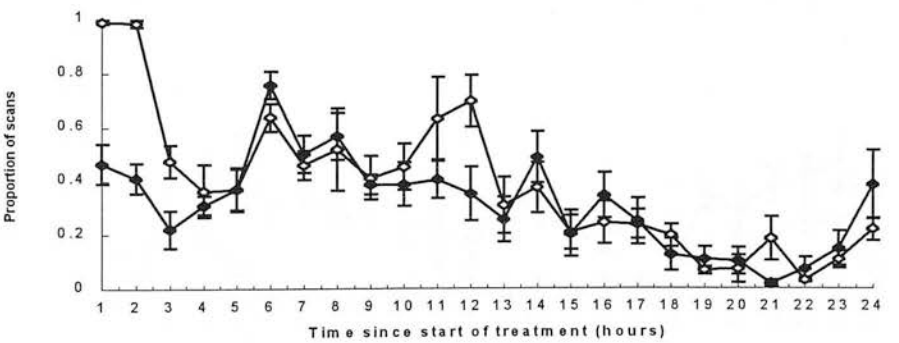
a) 6h food deprivation



b) 12h food deprivation

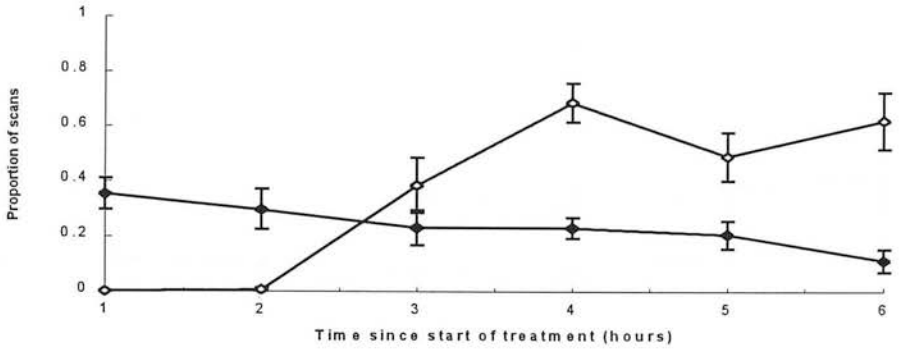


c) 18h food deprivation

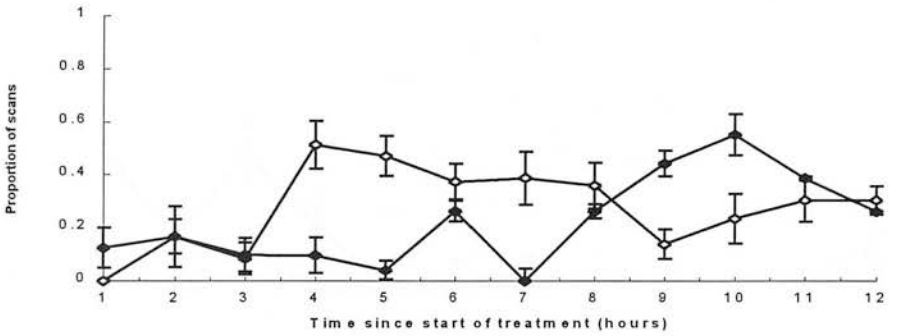


d) 24h food deprivation

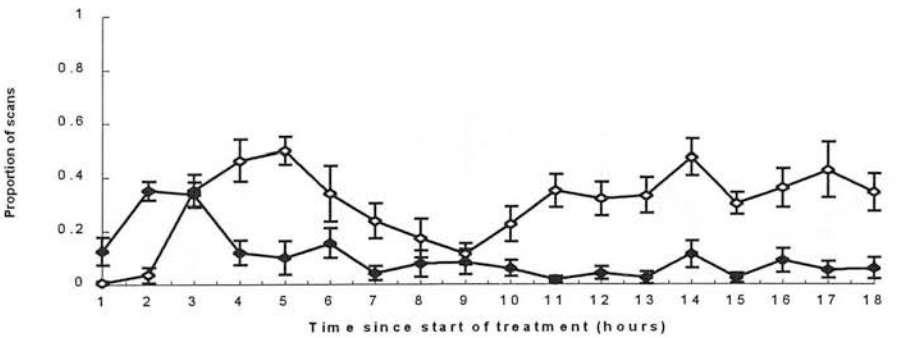
Figure 2.1. Effect of food deprivation of up to 24h on the mean proportion of scans spent standing during treatment (closed symbols) and the equivalent time pre-treatment (open symbols) (n=7). Vertical bars indicate s.e.



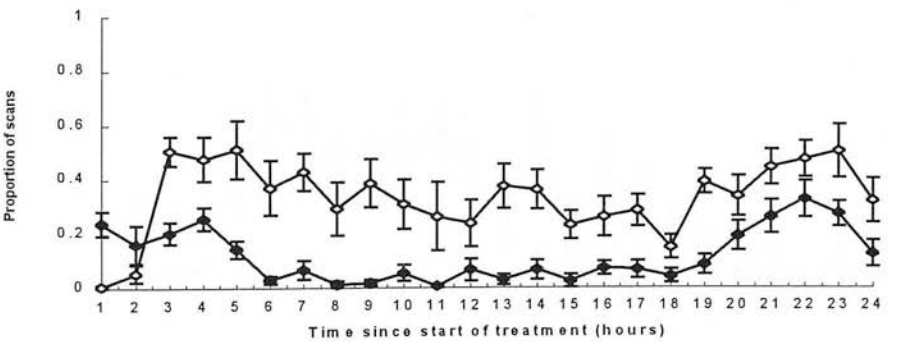
a) 6h food deprivation



b) 12h food deprivation

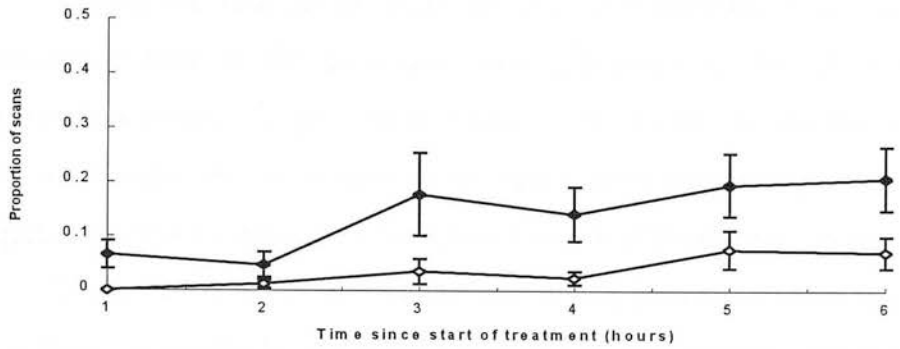


c) 18h food deprivation

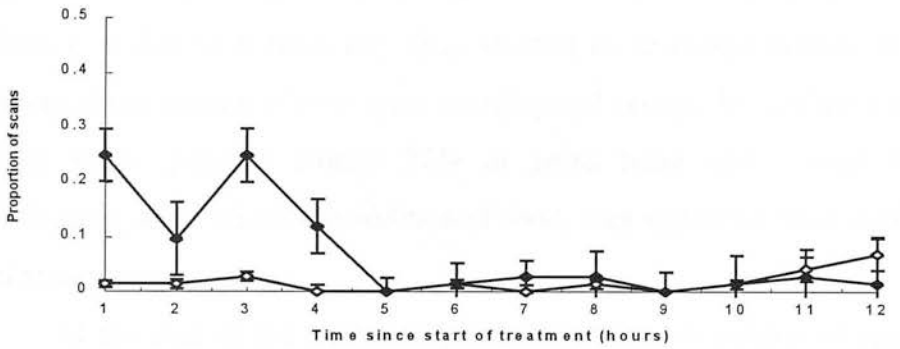


d) 24h food deprivation

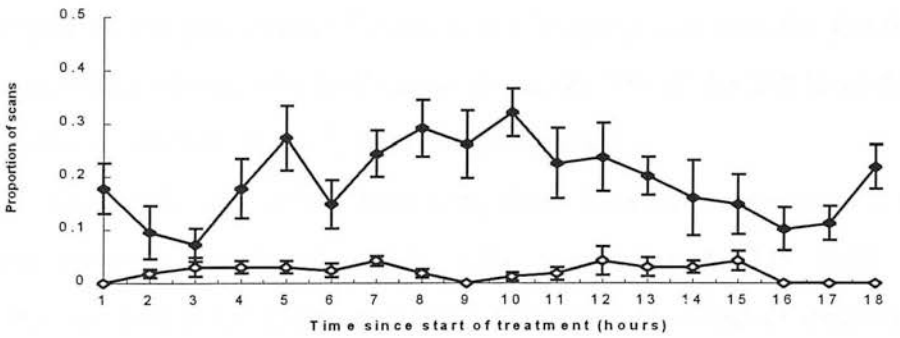
Figure 2.2. Effect of food deprivation of up to 24h on the mean proportion of scans spent ruminating during treatment (closed symbols) and the equivalent time pre-treatment (open symbols) (n=7). Vertical bars indicate s.e.



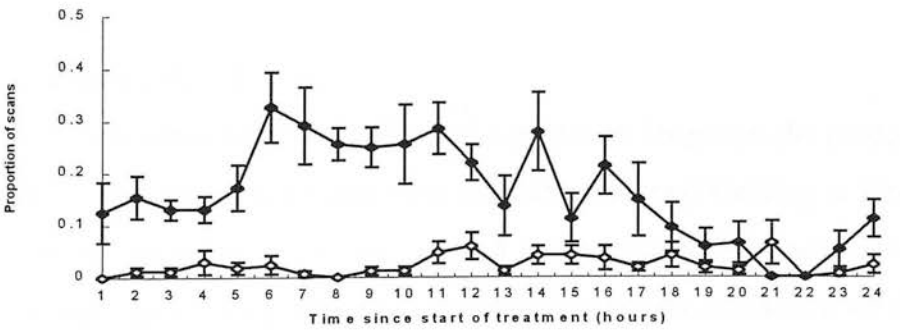
a) 6h food deprivation



b) 12h food deprivation



c) 18h food deprivation



d) 24h food deprivation

Figure 2.3. Effect of food deprivation of up to 24h on the mean proportion of scans spent foraging during treatment (closed symbols) and the equivalent time pre-treatment (open symbols) (n=7). Vertical bars indicate s.e.

During the first 2h of treatment, a greater proportion of scans were spent ruminating than at the equivalent time pre-treatment for all lengths of food deprivation except 12h ($p<0{\cdot}0001$, Figure 2.2). From 4h into the treatment, there was a decrease in the proportion of scans spent ruminating compared with the equivalent time pre-treatment during each length of food deprivation.

The initial increase in lying and ruminating (and the corresponding decrease in standing) can mainly be accounted for by the feeding regime. The time that all food deprivations began was the time when on non-treatment days, the sheep were fed. When provided with fresh hay, they showed an increased interest in the food and increased the amount of time spent standing and eating. Immediately before this, the sheep were spending around 70% of scans lying and around 50% of scans ruminating, and without the addition of food, they appear to have continued in these behaviours.

At the start of the treatment period, a greater proportion of scans were spent foraging than at the equivalent time pre-treatment ($p<0{\cdot}0001$, Figure 2.3). The increase in the proportion of scans spent foraging was seen for the duration of the treatment for 6h and 18h food deprivations, for 17h of the 24h food deprivation, but only for the first 4h of the 12h food deprivation.

During the first 18h of treatment, sheep took more steps than at the equivalent time pre-treatment (median (Q1, Q3): pre-treatment: 738 (636, 954); during treatment: 764 (492, 1289), $p<0{\cdot}01$). There was no effect of deprivation length or time on the frequency of butting during treatment.

Post-treatment

There was a significant effect of deprivation length on the proportion of scans spent performing all behaviours except ruminating and walking, a significant effect of time on the proportion of scans spent performing all behaviours except walking, and a significant effect of the time*deprivation length interaction on the proportion of scans spent performing all behaviours except foraging and walking during the first 12h post-treatment. During the second 12h post-treatment, there was a significant effect of deprivation length on the proportion of scans spent performing all

behaviours except foraging and walking. There were significant effects of time on the proportion of scans spent performing all behaviours except other behaviours and of time*deprivation length on the proportion of scans spent performing all behaviours except other behaviours and walking. Table III shows the mean percentages spent performing each behaviour during the two 12h post-treatment periods and the equivalent times pre-treatment.

Table III. Mean percentage of scans (s.e.) spent performing each behaviour post-treatment and during the equivalent time pre-treatment (n=7).

Length of deprivation(h)	1st 12h pre-treatment	2nd 12h pre-treatment	1st 12h post-treatment	2nd 12h post-treatment
Lying				
6	50.0(4.26)	59.9(2.42)	45.5(4.55)	65.5(3.00)
12	77.0(3.59)	36.5(2.33)	53.5(4.09)	49.2(2.25)
18	62.4(1.42)	62.7(4.63)	40.1(3.66)	62.6(4.63)
24	41.0(2.63)	79.3(3.02)	34.3(4.24)	71.1(4.72)
Standing				
6	48.5(4.30)	37.7(1.84)	54.4(4.48)	34.3(2.93)
12	21.8(3.65)	62.6(2.32)	46.3(4.17)	49.2(2.26)
18	36.8(1.33)	34.6(4.58)	60.2(3.86)	36.2(4.35)
24	58.3(2.71)	18.8(3.37)	65.2(4.29)	28.6(4.77)
Walking				
6	0.5(0.15)	0.5(0.21)	0.2(0.10)	0.2(0.10)
12	0.6(0.28)	0.9(0.18)	0.3(0.10)	0.2(0.10)
18	0.9(1.83)	0.5(0.13)	0.4(0.19)	0.5(0.40)
24	0.8(2.78)	0.9(0.27)	0.5(0.13)	0.3(0.16)
Eating				
6	31.4(3.96)	24.6(2.30)	44.0(2.94)	24.9(1.98)
12	5.4(0.44)	48.4(1.54)	31.7(1.98)	37.8(1.99)
18	25.4(1.18)	22.1(3.14)	45.9(2.47)	26.7(3.47)
24	37.5(2.06)	5.9(1.62)	47.3(2.64)	11.1(2.19)
Foraging				
6	3.7(1.52)	2.6(1.09)	1.1(0.62)	0.2(0.20)
12	3.4(1.57)	1.7(0.67)	1.2(0.54)	0.9(0.32)
18	1.4(0.34)	1.9(0.51)	1.4(0.53)	1.0(0.55)
24	1.9(0.65)	2.6(0.51)	0.3(0.14)	3.3(1.58)
Ruminating				
6	32.0(2.33)	42.9(1.96)	31.6(2.75)	41.0(2.24)
12	38.8(4.30)	27.2(1.94)	36.0(4.34)	35.5(2.35)
18	38.1(2.99)	30.6(1.58)	39.2(2.00)	38.6(1.70)
24	32.0(3.45)	34.6(2.77)	32.8(1.82)	38.7(3.72)
Other				
6	0.3(0.14)	0.7(0.32)	0.4(0.11)	0.3(0.13)
12	0.2(0.07)	0.8(0.14)	0.5(0.20)	0.4(0.15)
18	0.4(0.11)	0.4(0.12)	0.7(0.20)	0(0)
24	1.3(0.33)	0.1(0.10)	14.4(0.43)	0.2(0.07)

For the first 5h post-treatment, sheep spent a greater proportion of scans eating than at the equivalent time pre-treatment. This was seen after 6h, 12h and 18h food deprivation ($p<0{\cdot}0001$, Figure 2.4a-c). After 24h food deprivation, there was no difference in the proportion of scans spent eating during the first 4h post-treatment compared with the equivalent time pre-treatment. These sheep spent a greater proportion of scans eating than at the equivalent time pre-treatment from 4-8h post-treatment (Figure 2.4d). After the first 12h post-treatment, there was little difference compared with the equivalent time pre-treatment in the proportion of scans spent eating for all deprivation lengths. There was no difference in the proportion of scans spent eating during the first hour post-treatment between deprivation lengths, but for the remaining post-treatment period there were differences between the deprivation lengths ($p<0{\cdot}0001$). By 5h post-treatment the proportion of scans being spent eating by all groups was less than 0·5.

The length of food deprivation was a good predictor of post-treatment behaviour for the proportion of scans spent eating ($p<0{\cdot}05$) and foraging ($p<0{\cdot}05$) during the second 12h post-treatment (Table IV). The proportion of scans spent eating during the second 12h post-treatment decreased as the length of the food deprivation increased, while the proportion of scans spent foraging increased as the length of food deprivation increased.

During the first 2h post-treatment, after the 6h, 12h and 18h food deprivation, sheep spent a lower proportion of scans ruminating than at the equivalent time pre-treatment ($p<0{\cdot}0001$, Figure 2.5a-c). There were few differences in the proportion of scans spent ruminating compared with the equivalent times pre-treatment after 24h food deprivation (Figure 2.5d), and all groups showed little change to the equivalent time pre-treatment during the second 12h post-treatment.

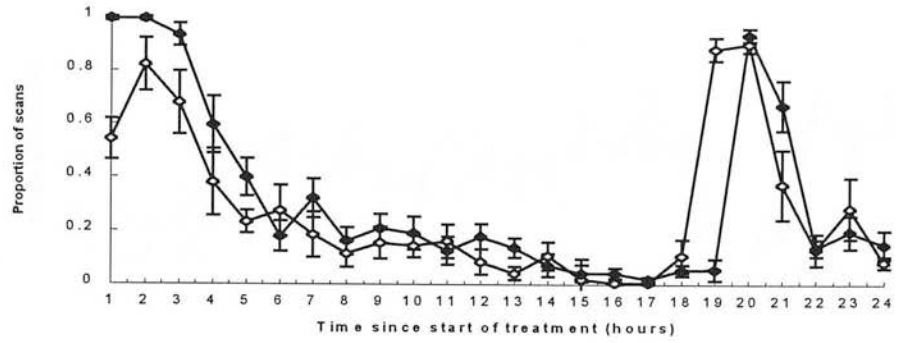
During the first 2h post-treatment, there was no effect of deprivation length on the proportion of scans spent lying or standing. During the next 10h, differences were seen between 24h food deprivation and 6h, 12h and 18h food deprivation in the proportion of scans spent standing ($p<0{\cdot}0001$) and lying ($p<0{\cdot}0001$). During the first hour after the 6h food deprivation, sheep spent a greater proportion of scans standing ($p<0{\cdot}0001$) and a lower proportion of scans lying ($p<0{\cdot}0001$) than at the equivalent

time pre-treatment. After the 12h and the 18h food deprivation this change in the proportion of scans spent standing and lying was seen for the first 5h post-treatment. After the 24h food deprivation no change in the proportion of scans spent standing and lying was seen for the first 2h post-treatment, but from 3-8h post-treatment sheep spent a greater proportion of scans standing and a lower proportion of scans lying than at the equivalent time pre-treatment. During the second 12h post-treatment, few differences were seen in the proportion of scans spent standing and lying compared with the equivalent time pre-treatment.

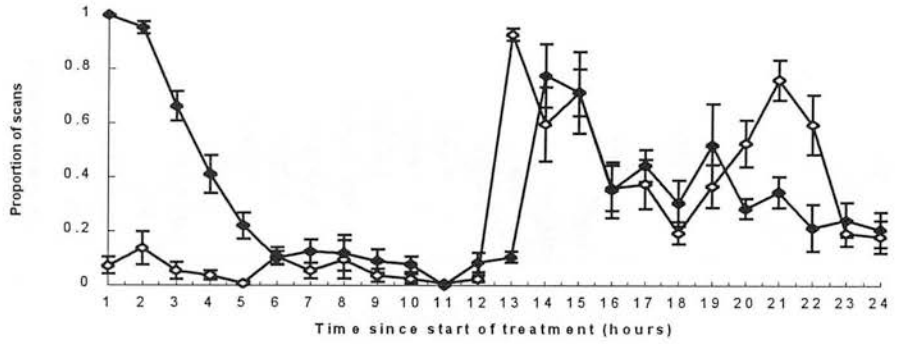
Table IV. Results of linear regression analysis on length of food deprivation and post-treatment behaviours (n=7).

		a	b	R^2	t	p
eat						
	1st 12h	0.363	0.0039	9.5	1.62	0.119
	2nd 12h	0.381	-0.0087	27.9	-3.11	0.005
forage						
	1st 12h	0.0164	-0.000426	5.3	-1.19	0.247
	2nd 12h	-0.0099	0.00159	20.1	2.51	0.019
ruminate						
	1st 12h	0.332	0.00096	0.7	0.43	0.669
	2nd 12h	0.393	-0.0051	0.3	-0.26	0.800
other						
	1st 12h	0.00025	0.000433	17.0	2.27	0.032
	2nd 12h	0.00347	-0.000104	6.0	-1.26	0.219
lie						
	1st 12h	0.551	-0.0077	18.1	-2.35	0.027
	2nd 12h	0.546	0.00467	6.8	1.35	0.188
stand						
	1st 12h	0.449	0.00766	17.6	2.31	0.029
	2nd 12h	0.453	-0.0048	7.3	-1.41	0.172
walk						
	1st 12h	0.00025	0.000199	15.9	2.18	0.039
	2nd 12h	0.00149	0.00009	1.4	0.61	0.546
butt						
	1st 12h	4.14	0.006	0.0	0.05	0.964
	2nd 12h	2.32	0.032	0.4	0.31	0.761
steps taken						
	1st 12h	418	10.1	15.8	2.16	0.040
	2nd 12h	532	-6.92	8.2	-1.49	0.148
drink						
	1st 12h	4.39	0.115	7.4	1.44	0.163
	2nd 12h	3.96	-0.1	26.0	-3.02	0.006

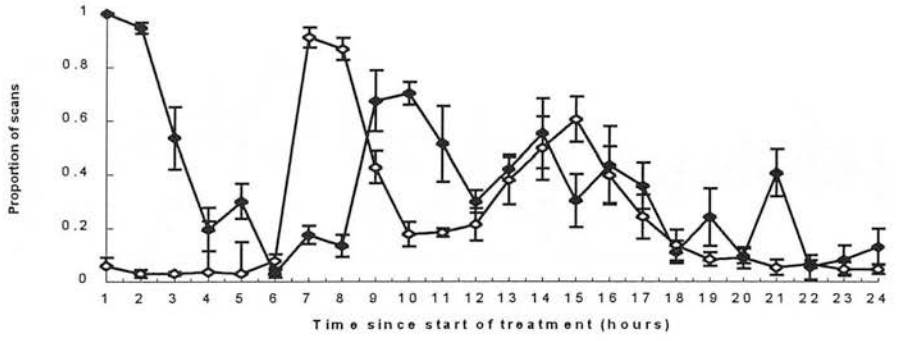
a=intercept (y=a+bx)
b=regression coefficient or slope
t=b/SE(b)
R^2=square of correlation coefficient
p=significance of the regression coefficient



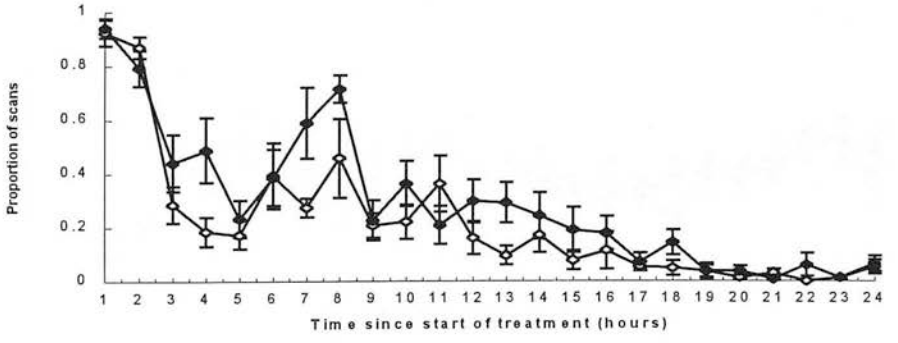
a) 6h food deprivation



b) 12h food deprivation

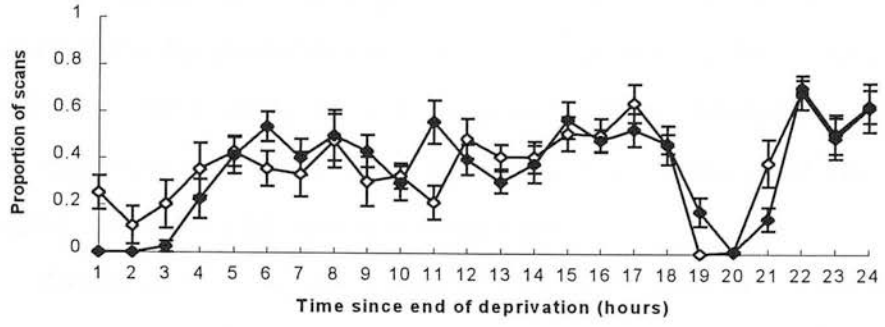


c) 18h food deprivation

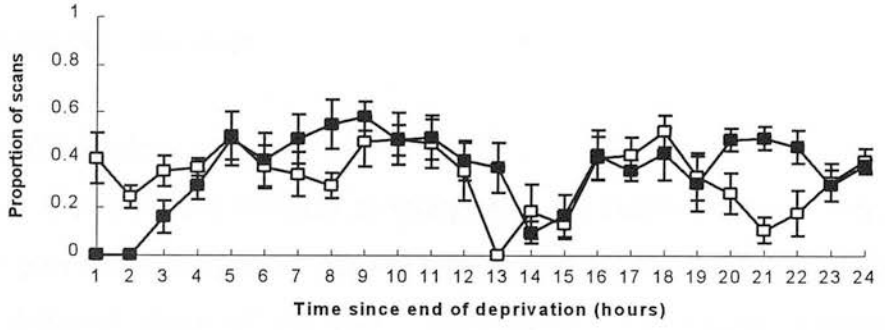


d) 24h food deprivation

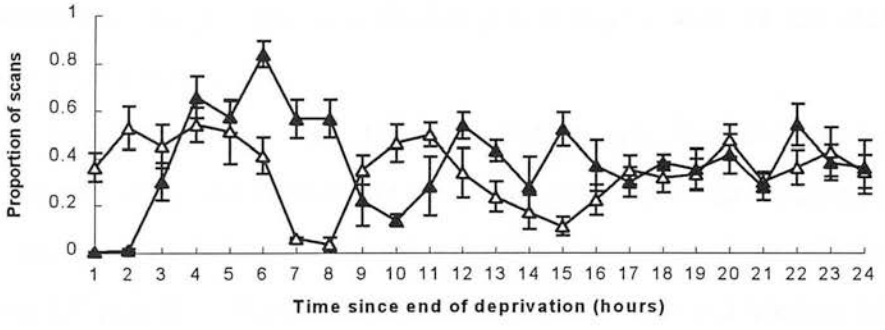
Figure 2.4. Effect of food deprivation of up to 24h on the mean proportion of scans spent eating post-treatment (closed symbols) and the equivalent time pre-treatment (open symbols) (n=7). Vertical bars indicate s.e.



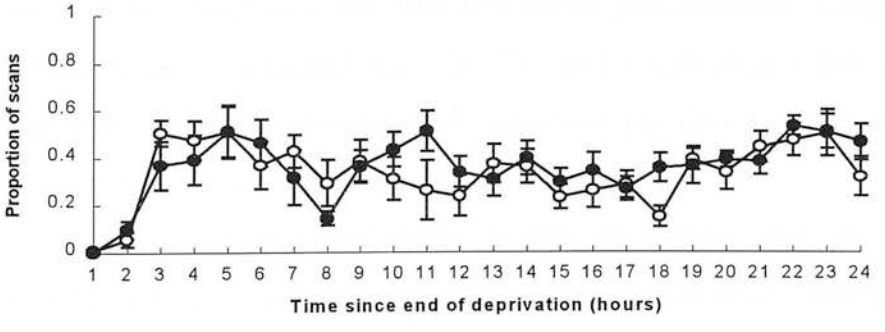
a) 6h food deprivation



b) 12h food deprivation



c) 18h food deprivation



d) 24h food deprivation

Figure 2.5. Effect of food deprivation of up to 24h on the mean proportion of scans spent ruminating post-treatment (closed symbols) and the equivalent time pre-treatment (open symbols) (n=7). Vertical bars indicate s.e.

The length of food deprivation was a good predictor of post-treatment behaviour for the proportion of scans spent standing ($p<0.05$), lying ($p<0.05$) and walking ($p<0.05$) during the first 12h post-treatment (Table IV). As the length of food deprivation increased, sheep spent a greater proportion of scans standing and walking and a lower proportion of scans lying.

During the first 6h post-treatment, sheep took more steps than at the equivalent time pre-treatment (median (Q1, Q3): pre-treatment: 226 (78, 324); post-treatment: 331 (265, 398), $p<0.01$). There was no effect of deprivation length or time on butting post-treatment.

2.4 Discussion

One potential problem in interpreting the results from this experiment is that the post-treatment periods for the different lengths of food deprivation commenced at different times of the day. This method was chosen because starting the treatments at the same time of day allowed direct comparisons during treatment, and ensured the sheep were in a similar physiological state at the start of the food deprivation periods.

Another problem with the methodology was the feeding regime. By not feeding the sheep *ad-libitum* hay pre-treatment, an increased interest in the hay was produced at feeding times each day. Other studies on sheep (Sibbald, 1997) and cattle (Winter & Hillerton, 1995) have reported increased feeding behaviour when provided with fresh food. This may explain the lack of difference in the proportion of scans spent eating over the first few hours post-treatment compared with the equivalent time pre-treatment after the 24h food deprivation. This is because the time at which food was returned to the pens was the time at which the sheep were normally fed pre-treatment.

As sheep will spend 9-11h/day feeding at pasture, with the time between feeding bouts being spent ruminating and resting (Lynch *et al.*, 1992), removing the time spent feeding is likely to have large effects on the rest of the daily behaviour. The increase in foraging behaviour and the number of steps taken during the periods

of food deprivation would suggest that a form of food-seeking behaviour is being performed. This would indicate an increased motivation to find food and feed.

The initial increase in feeding seen post-treatment irrespective of deprivation length agrees with changes seen after confinement on stationary/moving vehicles (Cockram *et al.*, 1996), which suggests a similar increase in the motivation to feed occurs after 6h, 12h and 18h food deprivation. Alternatively, the increase could be limited by the amount of food the sheep can physically consume. A number of studies have demonstrated effects of gut fill on voluntary food intake (Balch & Campling, 1962).

2.4.1 Effect of a period of food deprivation

Food deprivation resulted in a decrease in both water intake and the number of recorded drinking bouts. This agrees with previous work which found that sheep deprived of food drink little water (Hecker *et al.*, 1964). This is probably due in part to a lower requirement of water for saliva production during periods of food deprivation compared with when the sheep is feeding, with an estimated 2·7 ml saliva being swallowed with every 1g of dry food consumed (Stacy & Warner, 1966).

The decrease in the proportion of scans spent ruminating during treatment (following the increase seen during the first hour of treatment) has also been seen in transport studies (Cockram *et al.*, 1996). The decrease during treatment found in this study would suggest that the observed change in rumination during transport may have more to do with the lack of food than with exposure to adverse situations (Beilharz, 1985), in terms of novel environments, close confinement and vehicle motion. Studies involving the fasting of sheep for 56-72h found a rapid decline in the time spent ruminating, ceasing after 36h without food (Welch & Smith, 1968). As sheep ruminate following bouts of feeding (Lynch *et al.*, 1992), it may be that without the preceding bout of feeding, sheep will not ruminate or will ruminate to a lesser extent. However, Cockram *et al.* (1996) found that sheep confined on a stationary vehicle ruminated more than those confined on a moving vehicle, so it

would appear that the motion of the vehicles may also have an additional inhibitory effect during transport.

Rumination takes place to break down rumen contents, so if there are no new rumen contents, the amount of rumination will inevitably decrease leaving the sheep with more 'time to fill'. If the control of food intake involves rumen fill, this may explain the increase in this exploratory behaviour seen in this study, as the rumen will empty over time. However, this does not completely explain this change in behaviour, as the rumen takes several days to empty with a decrease in the number and strength of rumen contractions being observed during 18-24h fasting (Reid, 1963, in Church, 1979), but the change in behaviour was relatively quick to occur. Sheep fed concentrate foods that fulfil their nutritional requirements have been observed to develop 'abnormal' behaviours such as bar biting, slat chewing and wool eating, suggesting that the consumption of roughage is important to the sheep (Cooper *et al.*, 1995; Cooper & Jackson, 1996). If there is an effect of the physical action of eating on the voluntary food intake of sheep, this may explain the rapid increase in the proportion of scans spent foraging and the number of steps taken.

There seemed to be a point at which sheep 'gave up' looking for food, as during the 6h/18h food deprivation the increase in the proportion of scans spent foraging lasted the length of the deprivation, and during the 24h food deprivation, the increase lasted 17h. This switch in behaviour could be due to an increase in the tendency for lying to occur or to the removal of any inhibition to lying that occurred initially during the periods of food deprivation (McCleery, 1983). This change in tendency/inhibition may be caused by the natural circadian rhythm of the sheep. As most feeding bouts have been observed to occur between 07.00h and 22.00h (Lynch *et al.*, 1992), 18h into the 24h food deprivation corresponded to the time of day when less time was being spent feeding/looking for food. So, the sheep may become less motivated to find food at this time, or resting may become a higher priority.

Alternatively, as the food-seeking behaviour has failed to acheive its goal, the decrease in foraging behaviour and the increase in lying may be indicative of the sheep becoming increasingly passive in their relationship with the environment. Such inactivity has been described as 'learned helplessness' and has been associated

with depression (Wemelsfelder, 1993). However, this is usually associated with long-term housing in poor conditions with animals attempting to avoid novel stimuli. For example, in pigs, such inactivity is also associated with a lack of responsiveness to events (van Putten, 1980). As there was no difference in the activity of the sheep during this time compared with the equivalent time pre-treatment (in terms of the number of steps taken), the observed change in foraging behaviour would seem to be following the natural daily pattern of behaviour that was observed when sheep had access to food. The reduction in foraging behaviour may result from sheep having investigated the pens sufficiently to be convinced no food is present, and so they have stopped performing the behaviour.

2.4.2 Recovery of behaviour after a period of food deprivation

The initial lack of difference in behaviour post-treatment after the 24h food deprivation can be accounted for by the feeding regime, but the fact that from 4h post-treatment these sheep spent more time feeding than pre-treatment would suggest that the food deprivation has had more of an effect than just the excitatory effect of providing fresh food. An alternative feeding system that would allow *ad-libitum* access to hay and record the weight of hay left after each meal may decrease the effect of increased interest in fresh food and would provide more accurate measurements of post-deprivation food intakes over time.

The increase in feeding during the first 5h post-treatment is likely to be the cause of the decrease in rumination seen during this time, with almost 100% of scans being spent feeding over the first few hours post-treatment. This increase in feeding behaviour will also have resulted in the increased standing and decreased lying.

There was no change in the water intake post-treatment relative to pre-treatment intake, but it has been shown that sheep drink most of their requirement immediately after feeding (Ternouth, 1967), and so the water intake may have been affected during the first few hours post-treatment, but the 24h intakes measured in this study may have been too insensitive to pick this up. The increased number of recorded drinking bouts over the first 6h post-treatment would support this argument.

The increase in the number of steps recorded may have resulted from the increased frequency of drinking and the greater time spent eating, as the sheep may have walked to and from the water buckets more often, as well as moving around more whilst feeding.

2.5 Conclusions

Sheep perform increased amounts of behaviours that can be described as food-seeking during periods of food deprivation of up to 24h, and this may be indicative of an increased motivation to feed.

During the first hour post-treatment, there was no effect of deprivation length on eating, suggesting that initially, there is a similar motivation to feed after periods without food regardless of the length of time since the last meal. Post-treatment, all groups showed changes in behaviour from pre-treatment for a number of hours, showing that even without the additional stressors of confinement and transportation, periods of food deprivation of between 6h and 24h affect the behaviour of sheep and appear to cause an increased motivation to feed.

2.6 References

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CHAPTER 3 Effect of temperature and water provision on the behavioural and physiological responses of sheep during 24h food deprivation at a transport space allowance.

Abstract

A study was designed to investigate the effects of providing water during a 24h period of confinement without food at temperatures that can be experienced during export to mainland Europe. Behavioural observations and physiological measurements of fasting, dehydration and stress were made on 13-month-old sheep (liveweight 30kg), before, during and after confinement without food for 24h at a space allowance of 0·41m^2/sheep in climate chambers at temperatures of either 14°C or 21°C (n=6). One group in each chamber was provided with water during the confinement and visually isolated from a second group without access to water.

Some evidence of fasting was found during treatment, with plasma concentrations of free fatty acids increasing during treatment (pre-treatment: 0·30mmol/l; during treatment: 0·49mmol/l). Post-treatment hay and water intakes were greater (1·3kg, 3·4l) than intakes seen pre-treatment (1·2kg, 2·4l), with sheep spending a greater proportion of scans eating over the first 3-4h post-treatment (0·75) than during the equivalent time pre-treatment (0·49). The sheep became dehydrated post-treatment as they fed, with increases in plasma osmolality (301mosmol/l), and plasma concentrations vasopressin (0·90mmol/l) compared with pre-treatment values (osmolality: 295mosmol/l; vasopressin: 0·50mmol/l).

In this study, confinement at 21°C did not increase the risk of dehydration or increase the effect of fasting above that seen at 14°C. The consumption of large

quantities of dry feed after a 24h period of food deprivation has the potential to cause dehydration. This has implications for the lengths of lairage given to sheep when they are transported long distances, with an increased risk of dehydration if sheep are not given sufficient time to drink as well as feed.

3.1 Introduction

Legislation in both the UK and the EU has recognised public concerns about long distance transportation of animals, and requires the provision of a period of rest with food and water during long journeys. EU directive 95/29/EC (European Council, 1995) and the Welfare of Animals (Transport) Order (1997) allow a maximum journey time of 14h for sheep (on approved vehicles), which may be followed by a further 14h journey if at least 1h is allowed for watering and if necessary feeding.

When ruminants are deprived of food, less glucose is available from the gut or from gluconeogenesis in the liver, causing a reduction in the plasma glucose concentration. This reduction in plasma glucose concentration inhibits the release of insulin and increases the release of glucagon from the pancreas. These hormonal changes cause an increase in the adipose cell concentration of cAMP, resulting in the activation of hormone-sensitive lipase which catalyses the lipolysis of the adipose lipid tissue, releasing free fatty acids and glycerol. The increased oxidation of fatty acids also causes over production of beta-hydroxybutyrate in the liver (which can be utilised by tissues as an alternative energy fuel) (Warriss *et al.*, 1987; Bruss, 1989; Kaneko, 1989). These changes make decreases in the plasma concentration of glucose and increases in the plasma concentrations of free fatty acids and beta-hydroxybutyrate useful as potential indices of fasting in ruminants.

Water deficit causes a drop in fluid volume and a rise in the concentration of the extracellular fluid. A reduction in the extracellular fluid corresponds with a reduction in the volume of plasma and an increase in packed cell volume (PCV) and the concentration of total plasma proteins. The PCV is the proportion of the blood made up of red blood cells and is a measure of the relative heights of the red blood cells and the plasma in a sample after centrifuging. Total plasma protein

concentration is a measure of the amount of protein present in the plasma (Carlson, 1989; Houpt, 1993). Osmolality is the number of osmoles/kg water, where one osmole of any substance is 'the molecular weight in grams divided by the number of particles into which each molecule dissociates' (Carslon, 1989). Osmolality is mainly determined by the plasma concentration of sodium. Water restriction increases the plasma concentrations of sodium, potassium and chloride, which causes an increase in plasma osmolality. This change in osmolality is detected in the hypothalamus and stimulates the release of vasopressin (from the pituitary) and antidiuretic hormone (from the hypothalamus), which increase water reabsorption in the kidneys (Carslon, 1989). Increases in all of these biochemical measurements can be used as indicators of dehydration.

Journeys of 24h have been found to result in raised concentrations of blood metabolites (free fatty acids, beta-hydroxybutyrate and urea), indicating a mobilisation of body reserves in response to an energy deficit (Knowles *et al.*, 1995). After a 12h journey, sheep spend an increased amount of time feeding (Cockram *et al.*, 1996), suggesting they may be experiencing some form of 'hunger'.

The ambient temperature on lorries transporting sheep to France has been found to exceed 20°C for periods of up to 10h during journeys of 15h-34h, with sheep showing evidence of dehydration with increases in plasma total protein, albumin and osmolality during transport (Knowles *et al.*, 1994, 1996). However, individually penned sheep exposed to 48h of food and water deprivation at low (7°C) and high (35°C) ambient temperatures were found to be able to maintain normal plasma osmolality (Parrott *et al.*, 1996).

The close confinement that sheep are exposed to during transport is a potentially aversive stimulus. Studies in cattle have shown it to result in increased aggressive interactions (Kenny & Tarrant, 1982; Kondo *et al.*, 1989; Trunkfield & Broom, 1990). Tarrant *et al.*, (1988) found low space allowances during the transport of cattle produced a 'stress' response, with increased plasma concentrations of cortisol and glucose. The overall effect of cortisol is to supply glucose to the animal. It does this by stimulating the synthesis of a number of enzymes including those involved with gluconeogenesis in the liver (Rijnberk & Mol, 1989). The

release of cortisol from the adrenal cortex is stimulated by adrenocorticotropin hormone (ACTH) which is produced in the pituitary. ACTH release is stimulated by a number of hormones (including vasopressin and corticotropin releasing hormone) and physical and emotional stress (e.g. housing (Koelkebeck & Cain, 1984), surgery (Gould & Siegel, 1985), lactation (Gwazdauskas *et al.*, 1986)) (Ewbank, 1985; Mol & Rijnberk, 1989).

Space allowance during the transport of sheep has been shown to have an effect on behaviour (Cockram *et al.*, 1996), with sheep at lower space allowances (0·22m^2/sheep) spending less time lying down during a 12h journey. Further information is required on the combined effects of close confinement, elevated temperature and the absence of food and drinking water, similar to that experienced during transport, on the responses of sheep, in order to assess their impact on the welfare of sheep during transport.

This paper describes an investigation of the behavioural and physiological responses of sheep to 24h confinement at a space allowance typical of commercial transportation. In particular, it describes an assessment of how elevated temperatures and the provision of water during such a confinement affect the behaviour, biochemical measurements of the degree of mobilisation of body reserves and dehydration, and post-treatment food and water intake. The temperatures investigated were 14°C, a temperature experienced during transport within the UK, and 21°C, a temperature that can be experienced during export to mainland Europe (Knowles *et al.*, 1994, 1996). The elevated temperature was intended to be 25°C, but mechanical problems with the climate chamber resulted in the elevated temperature during treatment only reaching 21°C.

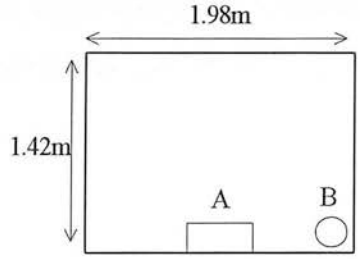
3.2 Material and methods

3.2.1 Animals and management

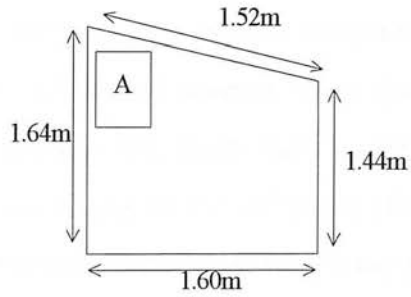
Twenty-four 13-month old Scottish Blackface ewes with an average liveweight of 30kg (s.e. 0·2) and an average fleece length of 93mm were used. They had previously been over-wintered on grass, with hay and sugar beet pulp supplement. One week before the start of the treatment, the sheep were individually housed in

pens with slatted floors at a space allowance of 2·81m^2/sheep (Figure 3.1a). The sheep were not visually isolated from surrounding pens, and the sheep were grouped according to treatment within the building. All sheep were individually marked with a stockmarker (Super Sprayline Stockmarker, Ritchey Tagg Ltd.) to enable identification of each sheep during the behavioural observations.

The sheep were provided with 2kg/day chopped hay (analysis: dry matter 915g/kg, acid-detergent fibre 418·84, crude protein 81·74, ash 78·71, organic mater 921·29, calcium 4·37 and phosphorus 2·62g/kg dry matter) at 09·00h and *ad-libitum* water.



a) Home pen. A- food trough (0·41m x 0·26m x 0·23m), B- water bucket (0·27m x 0·26m)



b) Treatment pen. A- water trough (0·6m x 0·4m x 0·66m).

Figure 3.1. Pens used to house sheep during experiment.

3.2.2 Blood sampling

Manual blood sampling was performed as described by Cockram *et al.* (1996). Three ml of blood was collected into 'Sarstedt' monovette tubes containing lithium heparin at 25h, 24h, 22h, 20h, 18h, 16h, 14h, 12h, 2h, and 1h before the start of treatment; at 2h intervals during the treatment, and at 3h, 6h, 9h, 12h, 18h, 24h, 30h,

and 36h after the end of the treatment. Half ml of the heparinised blood sample subsequently used for glucose analysis was immediately mixed in a tube containing fluoride. In addition, 5 ml of blood subsequently used for vasopressin analysis was collected into 'Sarstedt' monovette tubes containing 500 µl of 225 mM EDTA, 0·005 M 1,10 phenanthroline and 500 KI units aprotinin (Sigma, Poole) at 2h and 1h before the start of treatment; at 2h intervals during the treatment, and at 3h, 6h, 9h, 12h, 18h, 24h, 30h and 36h after the end of treatment. The tubes were stored in iced water during blood sampling and were then centrifuged at 5°C and the plasma removed and stored at -20°C.

3.2.3 Rectal temperature

The rectal temperature of the sheep was recorded 18h before the start of the treatment (15·00h) and 6h after the start of the treatment period (15·00h) using a clinical thermometer.

3.2.4 Procedure

At 09.00h, after the 24h of pre-treatment blood sampling, 12 sheep were moved into a climate chamber at a thermoneutral temperature of 14°C (s.e. 0·09) and 12 sheep were moved into an identical chamber at an elevated temperature of 21°C (s.e. 0·17). Within each chamber the sheep were penned in two groups of six on wood shavings at a space allowance of 0·41m^2/sheep (Figure 3.1b). One group in each chamber had constant access to water, and the other group had a empty covered trough of the same floor size. After 24h, the sheep were returned to their home pens which contained hay and water.

3.2.5 Behavioural observations

Direct observations of behaviours were made by scanning all sheep at 10 minute intervals from 09·00h-21·00h on the day prior to treatment and for 12h immediately following the treatment. Observations for each sheep were recorded on a Psion Organiser (LZ64) and analysed using behavioural observational software (Noldus 1990; Noldus & Potting, 1990).

The following behaviours were recorded:

standing (upright stationary posture)

moving (upright posture involving a change in location)

lying (recumbent posture with the body in contact with the floor)

eating (ingestion of food followed by jaw movements and swallowing)

ruminating (regurgitation, jaw movements and swallowing that is not immediately preceded by eating)

panting (rapid breathing with mouth open)

idling (performing no apparent behaviour)

other (performing any other behaviour not described above)

During the treatment period the behaviour was recorded using time lapse video equipment and analysed as described above for the direct observations, except that the incidence of drinking bouts (muzzles immersed in water for >5s) by each sheep was recorded continuously for the 24h treatment period.

3.2.6 Hay and water intake

The hay and water intakes of each sheep were recorded for the 24h pre-treatment and the first 24h post-treatment.

3.2.7 Environmental recordings

The air temperature in the climate chambers and the sheep pens was recorded at 5-minute intervals using Tinytalk Data Loggers (Orion Components Ltd., Chichester).

3.2.8 Laboratory analyses

Packed cell volume was measured using a Wifug haemicrofuge centrifuge. Plasma samples were analysed for vasopressin (McIntosh *et al.*, 1991; Smith *et al.*, 1990; 1992) (inter-assay CV was 0·12 and intra-assay CV was 0·10). Plasma cortisol concentration was assayed using the method described by Brooks & White (1990) with the modifications described by Cockram *et al.* (1996) (inter-assay CV was 0·11 and the intra-assay CV was 0·08).

The plasma concentrations of beta-hydroxybutyrate (Randox Laboratories Kit RB 1007, Randox Laboratories, Crumlin, Co. Antrim) were measured on a Bayer Diagnostics RA-2000 random access chemistry analyser (Bayer Diagnostics, Basingstoke) at 37°C. The plasma concentrations of sodium, potassium and chloride were measured on a Corning 644 electrolyte analyser using ion-selective reagents. Total plasma protein was measured by the biuret method, and free fatty acids (Randox Laboratories Kit FA/115S) and glucose (Randox Laboratories Kit GL 586), were measured on a Bayer Diagnostics RA-2000 random access chemistry analyser (Bayer Diagnostics, Basingstoke) at 37°C.

3.2.9 Statistical analysis

For each sheep, the proportion of scans spent in each behaviour was calculated for every hour of the observation periods. These proportions were used for statistical analysis (n=6). A repeated measures analysis of variance (Laird & Ware, 1982) using the mixed procedure within SAS version 6 (SAS Institute Inc., Cary USA) was used to examine the effects of temperature, presence of water and time on the mean proportion of scans spent performing each behaviour. Where there were interactions between temperature, presence of water and time, the differences between least-square means were examined by comparing the 12h pre-treatment period and the 12h post-treatment period. This analysis was repeated to examine the effects of temperature, presence of water and time on the biochemical measurements comparing : 1st 12h pre-treatment; 2nd 12h pre-treatment; 1st 12h during treatment; 2nd 12h during treatment; 1st 12h post-treatment; 2nd 12h post-treatment; 3rd 12h post-treatment. The analysis was also repeated to examine the effects of temperature, presence of water and time on the hay and water intakes for each sheep comparing intakes during the 24h post-treatment with the intakes during the equivalent 24h pre-treatment. The analysis was also repeated to examine the effects of temperature, presence of water and time on the rectal temperatures, comparing rectal temperature during treatment with the pre-treatment rectal temperature.

3.3 Results:

3.3.1 Hay and water intake

The mean hay intake post-treatment (1·3kg s.e. 0·03) was greater than pre-treatment (1·2kg s.e. 0·03, $p<0·05$), but there was no effect of either temperature or provision of water on the weight of hay consumed post-treatment.

Total water intake during the 24h treatment period was 3 litres for the group kept at 14°C (0·5l/sheep) and 4 litres for the group kept at 21°C (0·7l/sheep). Mean water intake post-treatment (3·4 litres s.e. 0·12) was higher than pre-treatment (2·4 litres s.e. 0·17, $p<0·001$). There was a significant interaction between temperature and the provision of water on the change in water intake ($p<0·01$). The group kept at 14°C without water and the group kept at 21°C with water had the largest changes in water intake (mean change in water intake: 14°C with water +0·4 litres s.e. 0·21; 14°C without water +1·6 litres s.e. 0·60; 21°C with water +1·5 litres s.e. 0·29; 21°C without water +0·6 litres s.e. 0·28).

3.3.2 Rectal temperatures

There was a significant decrease in rectal temperature during treatment (means: before 39·6°C s.e. 0·07; during 39·2°C s.e. 0·06, $p<0·0001$).

3.3.3 Measures of feed restriction:

Behaviour

During treatment

Figure 3.2 shows the proportion of scans spent ruminating over the whole treatment period for the four treatment groups. Over the first 12h of treatment, sheep kept at 14°C ruminated for a lower proportion of scans than those kept at 21°C (14°C 0·02, 21°C 0·09), but over the second 12h of the treatment period, similar amounts of rumination occurred at both temperatures (14°C 0·08, 21°C 0·07).

During the first 12h of the treatment period, similar proportions of scans were spent ruminating by sheep kept with and without water (with water 0·06, without water 0·05), but over the second 12h treatment period, while the amount of

rumination increased, the increase was greater for sheep kept with access to water than for those kept without access to water (with water 0·15, without water 0·07).

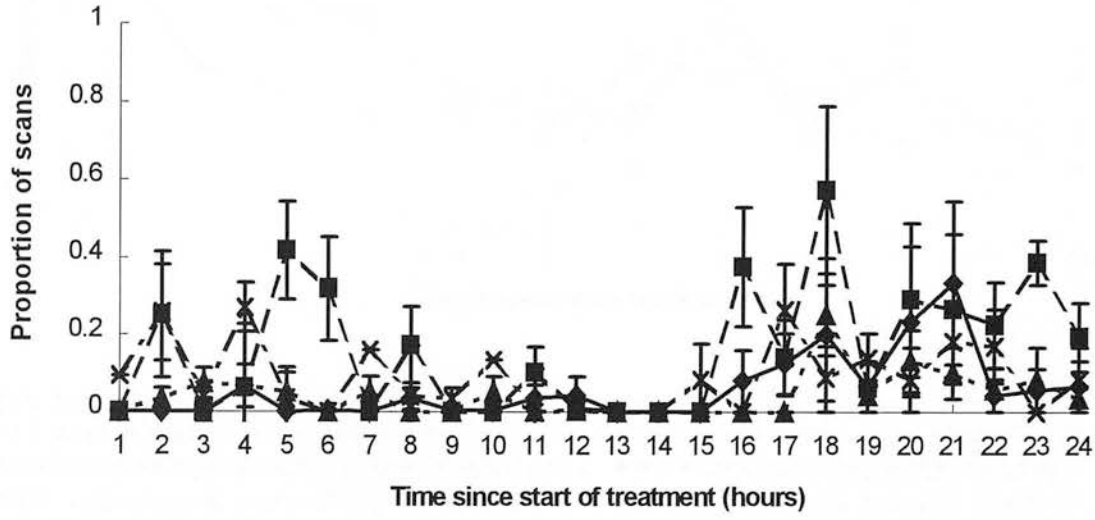


Figure 3.2. Effect of 24h close confinement at two air temperatures with or without access to water on the mean proportion of scans spent ruminating during the 24h treatment period (n=6). ◆- 14°C with access to water; ■- 21°C with access to water; ▲ -14°C without access to water; X- 21°C without access to water. Vertical bars show s.e.

Post-treatment

There were significant effects of time, and the interactions between time and the temperature of the climate chamber during treatment (temp), and between time, temp and the provision of water during treatment (water) on the proportion of scans spent eating. Figure 3.3 shows the proportion of scans spent eating over the pre-treatment and post-treatment periods for the four treatment groups. Sheep that had been kept at 21°C spent a greater proportion of scans eating during the first hour post-treatment than during the equivalent time pre- treatment ($p<0·01$). All groups spent a significantly greater proportion of scans eating from 2h-4h post-treatment than at the equivalent time pre-treatment ($p<0·01$).

From 4h post-treatment, eating and rumination behaviour were similar to that seen during the equivalent time pre-treatment.

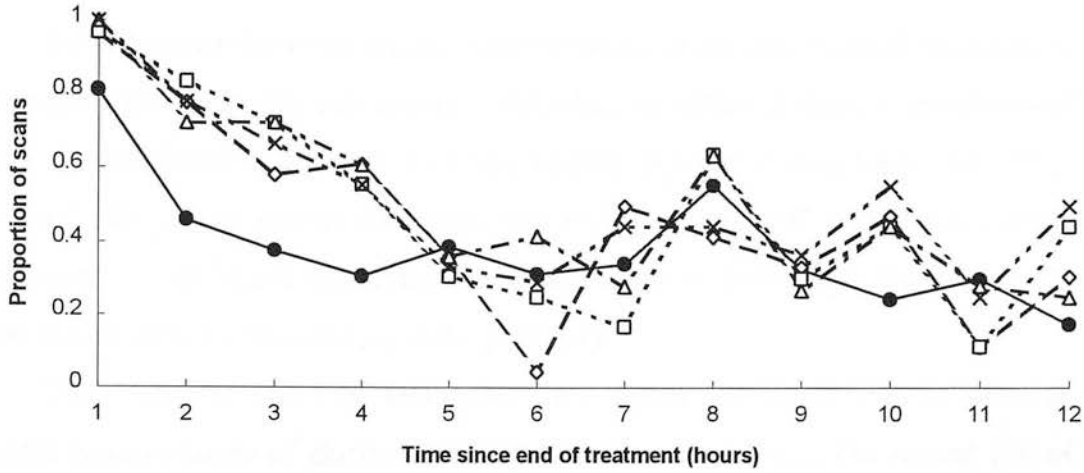


Figure 3.3. Effect of 24h close confinement at two air temperatures with or without access to water on the mean proportion of scans spent eating during the first 12h post-treatment (open symbols) and the equivalent time pre-treatment (closed symbol) (n=6). ●-pre-treatment averaged over all groups; ◊- 14°C with access to water; □- 21°C with access to water; Δ- 14°C without access to water; X- 21°C without access to water.

Table I. Mean plasma concentrations (s.e.) of beta-hydroxybutyrate (B-OH), free fatty acid (FFA), glucose and cortisol before, during and after treatment for sheep held for 24h at two temperatures with and without water (n=6).

	temp °C	water	Pre-treatment	During 1st 12h	treatment 2nd 12h	Post-treatment 1st 12h	2nd 12h	3rd 12h
B-OH	14°C	with	0·27	0·33	0·30	0·35	0·24	0·36
			(0·01)	(0·06)	(0·04)	(0·06)	(0·04)	(0·07)
mmol/l	14°C	without	0·24	0·23	0·26	0·23	0·29	0·40
			(0·04)	(0·03)	(0·07)	(0·05)	(0·08)	(0·10)
	21°C	with	0·20	0·21	0·20	0·20	0·16	0·22
			(0·03)	(0·03)	(0·03)	(0·03)	(0·01)	(0·04)
	21°C	without	0·27	0·19	0·18	0·18	0·19	0·18
			(0·04)	(0·03)	(0·02)	(0·01)	(0·02)	(0·02)
FFA	14°C	with	0·42	0·40	0·44	0·36	0·28	0·21
			(0·07)	(0·07)	(0·06)	(0·07)	(0·06)	(0·03)
mmol/l	14°C	without	0·31	0·46	0·49	0·34	0·23	0·25
			(0·05)	(0·06)	(0·07)	(0·05)	(0·04)	(0·04)
	21°C	with	0·25	0·54	0·67	0·33	0·22	0·17
			(0·05)	(0·07)	(0·08)	(0·08)	(0·04)	(0·03)
	21°C	without	0·20	0·42	0·49	0·27	0·13	0·14
			(0·07)	(0·10)	(0·08)	(0·06)	(0·06)	(0·06)
Glucose	14°C	with	3·2	3·4	3·2	3·4	3·4	3·5
			(0·09)	(0·04)	(0·09)	(0·08)	(0·11)	(0·10)
mmol/l	14°C	without	3·4	3·6	3·4	3·6	3·4	3·5
			(0·10)	(0·09)	(0·08)	(0·08)	(0·04)	(0·17)
	21°C	with	3·4	3·5	3·3	3·5	3·5	3·6
			(0·03)	(0·07)	(0·08)	(0·05)	(0·04)	(0·09)
	21°C	without	3·4	3·5	3·2	3·6	3·4	3·4
			(0·07)	(0·06)	(0·05)	(0·07)	(0·05)	(0·06)

Biochemical measures of feed restriction

Table I shows the mean plasma concentrations of the biochemical measures of feed restriction during the experiment. There was no effect of time, or provision of water on the plasma concentration of beta-hydroxybutyrate during treatment. Over the final 12h post-treatment, those that had been kept at 14°C had higher plasma concentrations of beta-hydroxybutyrate (0·4mmol/l s.e. 0·05) than those that had been kept at 21°C (0·2mmol/l s.e. 0·05, $p<0·001$).

There was no effect of temperature on plasma concentrations of glucose. Plasma concentrations of glucose were significantly lower during the second 12h of the treatment period (3·3 mmol/l s.e. 0·05) than during the first 12h of the treatment period (3·5 mmol/l s.e. 0·05, $p<0·0001$).

Plasma concentrations of free fatty acids increased during treatment. At 10h from the start of treatment, plasma concentrations of free fatty acids were significantly higher than at the equivalent time pre-treatment ($p<0·0001$) (Figure 3.4). Post-treatment, the plasma concentrations of free fatty acids fell to below those seen during treatment ($p<0·0001$), and this decrease continued over the whole 36h post-treatment period.

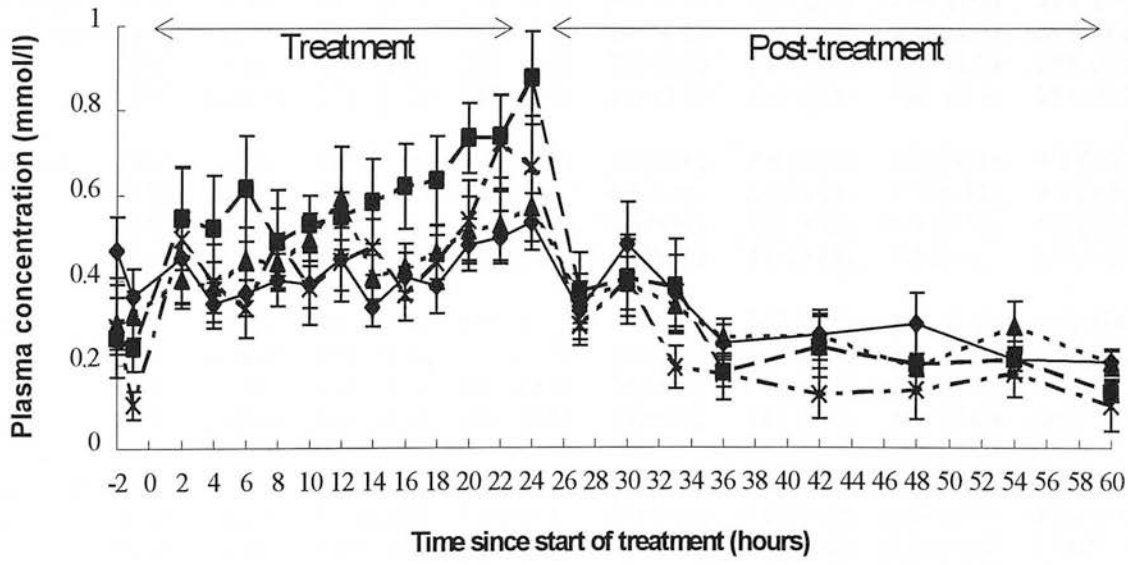


Figure 3.4. Effect of 24h close confinement at two air temperatures with or without access to water on the mean plasma concentration of free fatty acids over the treatment and post-treatment periods (n=6). Vertical bars show s.e. For legends see Figure 3.2.

3.3.4 Measures of water restriction

Behaviour

No panting was observed during any observation period. The total number of drinking bouts observed in all groups during the first 12h of the treatment period (34) was greater than during the second 12h treatment period (6). Over the first 12h treatment period, the number of drinking bouts was similar at each temperature (14°C: 16, 21°C: 18). Over the second 12h treatment period, the number of drinking bouts was low (21°C: 5, 14°C: 1).

Table II. Mean (s.e.) packed cell volume (PCV), and plasma concentrations of total protein (TP), osmolality, potassium, sodium, vasopressin and chloride before, during and after treatment for sheep held for 24h at two temperatures with and without water (n=6).

	temp °C	water	Pre treatment	During 1st 12h	treatment 2nd 12h	Post 1st 12h	treatment 2nd 12h	3rd 12h
PCV	14°C	with	28·2(0·71)	29·0(0·82)	27·7(0·67)	30·8(0·69)	30·3(0·74)	20·1(0·44)
	14°C	without	28·3(1·00)	27·9(1·13)	26·4(0·89)	31·5(1·40)	29·3(1·16)	26·7(1·02)
	21°C	with	29·7(0·66)	30·8(0·82)	28·8(0·83)	32·5(1·14)	31·3(1·14)	28·3(1·21)
	21°C	without	29·0(0·83)	28·9(1·15)	27·8(1·09)	30·9(1·06)	30·9(1·18)	28·0(1·09)
TP	14°C	with	78·7(2·42)	80·5(2·55)	77·4(3·54)	76·8(3·48)	76·2(2·57)	78·1(3·74)
g/l	14°C	without	78·5(1·35)	78·9(1·81)	75·7(1·75)	76·4(2·02)	78·7(1·90)	76·7(1·39)
	21°C	with	79·2(1·67)	81·1(4·64)	74·6(2·01)	79·8(4·11)	80·5(4·07)	78·1(4·75)
	21°C	without	76·7(0·96)	76·3(1·15)	73·6(1·13)	74·6(1·39)	75·7(1·53)	73·3(1·76)
Osmolality	14°C	with	295 (1·4)	294 (1·2)	297 (1·3)	307 (2·7)	296 (1·2)	292 (0·7)
mosmol/kg	14°C	without	293 (1·2)	293 (1·0)	297 1·1)	310 (1.1)	294 (1·5)	291 (1·0)
	21°C	with	297 (2·4)	297 (1·6)	300 (1·2)	301 (0·6)	297 (1·0)	292 (0·4)
	21°C	without	294 (1·3)	295 (1·1)	299 (1·0)	304· (2·8)	302 (3·1)	294 (0·9)
Potassium	14°C	with	4·7 (0·15)	4·2 (0·08)	4·6(0·20)	4·8 (0·22)	4·6 (0·14)	4·1 (0·17)
mmol/l	14°C	without	4·5 (0·52)	4·1 (0·17)	4·4 0·34)	5·0 (0·45)	4·5 (0·32)	4·1 (0·31)
	21°C	with	4·4 (0·25)	4·4 (0·20)	4·5 0·25)	4·8 0·32)	4·4 (0·16)	4·0 (0·16)
	21°C	without	4·3 (0·15)	4·0 (0·11)	4·4(0·16)	4·6 (0·21)	4·3 (0·14)	3·7 (0·10)
Sodium	14°C	with	144 (1·3)	145 (0·7)	144(1·2)	145 (0·6)	144 (0·8)	143 (1·0)
mmol/l	14°C	without	145 (1·7)	144 (1·2)	144(1·3)	147 (1·2)	145 (1·4)	144 (1·2)
	21°C	with	145 (1·2)	144 (0·7)	143(0·8)	146 (0·6)	144 (0·4)	143 (0·3)
	21°C	without	144 (0·7)	142 (0·6)	142(0·6)	147 (1·3)	144 (1·5)	143 (0·5)
Vasopressin	14°C	with	0·50(0·15)	0·41(0·11)	0·38(0·07)	0·91(0·13)	0·56(0·08)	0·52(0·07)
nmol/l	14°C	without	0·34(0·07)	0·46(0·11)	0·41(0·09)	0·89(0·14)	0·54(0·07)	0·56(0·09)
	21°C	with	0·59(0·05)	0·81(0.20)	0·65(0·21)	1·07(0·10)	0·56(0·03)	0·58(0·07)
	21°C	without	0·58(0·11)	0·54(0·14)	0·55(0·17)	1·72(0·56)	0·83(0·35)	0·80(0·16)
Chloride	14°C	with	109 (1·4)	109 (0·7)	110 (0·5)	113 (1·2)	111 (0·6)	111 (0·5)
mmol/l	14°C	without	109 (1·4)	109 (1·0)	109 (1·2)	115 (1·5)	112 (1·3)	109 (1·6)
	21°C	with	109 (0·9)	108 (1·0)	108 (0·8)	114 (0·4)	111 (0·6)	109 (0·5)
	21°C	without	108 (0·9)	107 (0·7)	109 (0·6)	115 (1·1)	111 (0·8)	110 (0·2)

Biochemical measures of water restriction

Table II shows the mean values and plasma concentrations of the biochemical measure of dehydration during the experiment. During the treatment period there were few significant effects of either temperature or the provision of water on the biochemical measures of dehydration. During the treatment period, the packed cell volume was lower (28·4 s.e. 0·54) than pre-treatment (34·6 s.e. 0·54)($p<0·0001$).

During the first 12h post-treatment, the mean plasma concentrations of sodium (147 mmol/l s.e. 0·62) was greater than pre-treatment (145 mmol/l s.e. 0·62) ($p<0·0001$). Figure 3.5 shows the plasma concentrations of vasopressin over the whole treatment period for the four treatment groups. During the first 24h post-treatment, plasma osmolality (296 mosmol/kg s.e. 1·02) and plasma concentrations of vasopressin (1·0 mmol/l s.e. 0·11) were greater than pre-treatment (osmolality: 295 mosmol/kg s.e. 1·02; vasopressin: 0·5 mmol/l s.e. 0·11) ($p<0·0001$).

The packed cell volume increased to 32·9 at 3h post-treatment, but over the remaining samples post-treatment it fell to levels seen during treatment. During the first 12h post-treatment, the packed cell volume (31·4 s.e. 0·54) was lower than pre-treatment (35·6 s.e. 0·54) ($p<0·0001$). During the first 24h post-treatment, the plasma concentration of total protein (77·1 mmol/l s.e. 1·5) was lower than pre-treatment (78·7 s.e. 1·5) ($p<0·0001$).

During the first 12h post-treatment, the plasma vasopressin concentration in the group that had been kept at 21°C without water (1·3 nmol/l s.e. 0·22) was greater than in the group that had been kept at 21°C with water (1·0 nmol/l s.e. 0·22)($p<0·05$). During the second 12h post-treatment period, the plasma concentration of vasopressin (0·8 nmol/l s.e. 0·22) and the plasma osmolality (302 mosmol/kg s.e. 2·0) in the group that had been kept at 21°C without water was greater than in the groups kept at either 21°C with water (0·6 nmol/l s.e. 0·22; 294 mosmol s.e. 2·0) or 14°C without water (0·6 nmol/l s.e. 0·22; 297 mosmol/kg s.e. 2·0)($p<0·05$). During the first 12h post-treatment, the plasma osmolality was greater in those that had been kept at 14°C with water (306 mosmol/kg s.e. 2·0) than in those that had been kept at either 21°C with water (301 mosmol/kg s.e. 2·0) or 14°C without water (301 mosmol/kg s.e. 2·0)($p<0·01$).

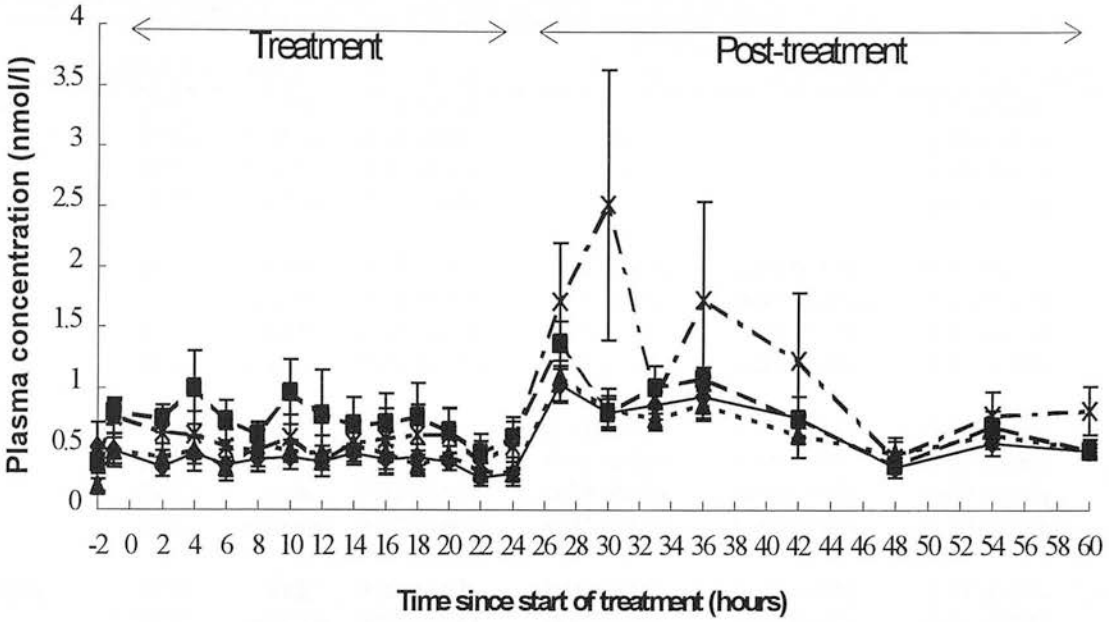


Figure 3.5. Effect of 24h close confinement at two air temperatures with or without access to water on the mean plasma concentration of vasopressin over the treatment and post-treatment periods (n=6). Vertical bars show s.e. For legends see Figure 3.2.

3.3.5 Biochemical measures of stress

Table III shows the mean plasma concentration of cortisol during the experiment. There were no effects of temperature or water provision on the plasma concentration of cortisol during the treatment and post-treatment periods.

Table III. Mean plasma concentrations (s.e.) of cortisol before, during and after treatment for sheep held for 24h at two temperatures with and without water (n=6).

	temp °C	water	Pre-treatment	During 1st 12h	treatment 2nd 12h	Post-treatment 1st 12h	2nd 12h	3rd 12h
Cortisol	14°C	with	34·5	44·0	32·8	36·2	35·3	48·7
			(7·05)	(9·29)	(7·06)	(7·80)	(5·93)	(5·26)
nmol/l	14°C	without	36·8	30·2	33·9	30·7	35·2	27·0
			(9·40)	(5·95)	(3·68)	(6·60)	(9·66)	(6·84)
	21°C	with	41·9	44·8	48·9	47·1	41·8	28·8
			(13·90)	(9·41)	(10·1)	(12·00)	(10·40)	(8·47)
	21°C	without	34·5	33·2	35·0	46·9	41·1	24·0
			(3.82)	(4·34)	(5·55)	(3·50)	(5·37)	(3·82)

Table IV. Mean proportion (s.e.) of scans spent performing behaviours before, during and after treatment for sheep held for 24h at two temperatures with and without water (n=6).

				During treatment		
	temp(C)	water	Pre-treatment	1st 12h	2nd 12h	Post-treatment
eating	14°C	with	0·38 (0·03)	-	-	0·46 (0·04)
	14°C	without	0·44 (0·04)	-	-	0·50 (0·06)
	21°C	with	0·34 (0·05)	-	-	0·50 (0·04)
	21°C	without	0·37 (0·04)	-	-	0·52 (0·03)
idling	14°C	with	0·25 (0·03)	0·93 (0·02)	0·88 (0·02)	0·13 (0·03)
	14°C	without	0·25 (0·02)	0·97 (0·01)	0·87 (0·003)	0·13 (0·02)
	21°C	with	0·33 (0·05)	0·76 (0·05)	0·71 (0·04)	0·15 (0·04)
	21°C	without	0·32 (0·05)	0·85 (0·03)	0·87 (0·03)	0·19 (0·04)
lying	14°C	with	0·40 (0·03)	0·38 (0·04)	0·70 (0·04)	0·42 (0·06)
	14°C	without	0·30 (0·03)	0·14 (0·05)	0·40 (0·07)	0·34 (0·04)
	21°C	with	0·31 (0·03)	0·38 (0·07)	0·50 (0·07)	0·33 (0·05)
	21°C	without	0·33 (0·04)	0·06 (0·04)	0·48 (0·07)	0·29 (0·02)
moving	14°C	with	0·02 (0·01)	0·09 (0·02)	0·02 (0·01)	0·02 (0·01)
	14°C	without	0·03 (0·01)	0·10 (0·02)	0·03 (0·01)	0·01 (0·01)
	21°C	with	0·04 (0·02)	0·03 (0·01)	0·01 (0·01)	0·04 (0·02)
	21°C	without	0·04 (0·02)	0·03 (0·01)	0·002 (0·002)	0·03 (0·01)
other	14°C	with	0·01 (0·003)	0·05 (0·02)	0·03	0·01 (0·01)
	14°C	without	0·01 (0·003)	0	0·05 (0·01)	0·002 (0·002)
	21°C	with	0	0·09 (0·02)	0·02	0·005 (0·005)
	21°C	without	0·002 (0·002)	0·09 (0·03)	0·04 (0·02)	0·002 (0·002)
ruminating	14°C	with	0·39 (0·05)	0·02 (0·01)	0·09 (0·02)	0·37 (0·06)
	14°C	without	0·33 (0·02)	0·03 (0·01)	0·08 (0·01)	0·32 (0·04)
	21°C	with	0·35 (0·03)	0·15 (0·05)	0·25 (0·04)	0·32 (0·03)
	21°C	without	0·35 (0·03)	0·06 (0·01)	0·09 (0·02)	0·27 (0·02)
standing	14°C	with	0·57 (0·04)	0·54 (0·03)	0·28 (0·03)	0·57 (0·06)
	14°C	without	0·68 (0·03)	0·76 (0·05)	0·56 (0·06)	0·65 (0·03)
	21°C	with	0·66 (0·02)	0·59 (0·08)	0·48 (0·06)	0·63 (0·04)
	21°C	without	0·64 (0·05)	0·91 (0·04)	0·52 (0·07)	0·67 (0·01)

3.3.6 Behaviour

During treatment

Resting behaviour

Table IV shows the mean proportion of scans spent in each recorded behaviour during the experiment. The proportion of scans spent lying and standing during the whole 24h treatment period were similar for both climate chambers. During the first 12h of the treatment period, sheep kept without access to water spent a greater proportion of scans standing (0·83) and a lower proportion of scans lying (0·10) than

those with access to water (standing: 0·56, lying: 0·38). The amount of lying increased during the second 12h treatment period, sheep with access to water continued to spent more time lying (0·59) than sheep with no access to water (0·44).

The proportion of scans spent moving were greater during the first 12h treatment period (0·06) than over the second 12h treatment period (0·02). Sheep kept at 14°C spent a greater proportion of scans moving (0·06) than sheep kept at 21°C (0·02) during the first 12h treatment period.

Other behaviours

A large amount of time was spent idling during the treatment period (0·86), with sheep kept at 14°C idling more during the first 12h treatment period (0·96) than those kept at 21°C (0·83). During the second 12h treatment period, sheep without access to water idled more (0·89) than those with access to water (0·76)

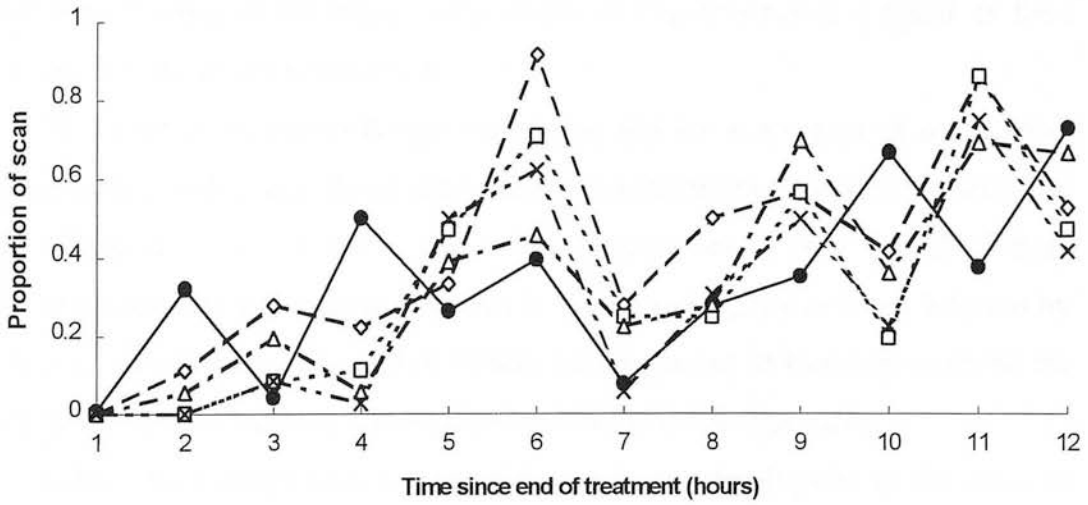


Figure 3.6. Effect of 24h close confinement at two air temperatures with or without access to water on the mean proportion of scans spent lying during the first 12h post-treatment compared with the equivalent time pre-treatment (n=6). For legends, see Figure 3.3.

Post-treatment

Resting behaviour

Figure 3.6 shows the mean proportion of scans spent lying during the pre- and post-treatment periods for all four treatment groups. In the first hour post-treatment there was no difference in the proportion of scans spent standing or lying compared with the equivalent hour pre-treatment. Differences between post-treatment and pre-treatment for both standing ($p<0{\cdot}0001$) and lying ($p<0{\cdot}0001$) were seen for most of the remaining 11h.

3.4 Discussion

The temperatures used in this study were not as diverse as was intended, due to mechanical failures in the hot climate chamber. However, the range is comparable to the change in temperature to which sheep can be exposed, particularly if moved from Scotland to the south of England.

The changes in rectal temperature were small and there was no evidence of either hyperthermia at the higher temperature or hypothermia as a result of food deprivation at the lower temperature.

The increase in hay intake post-treatment and the proportion of scans spent feeding agrees with results found after 12h transport/confinement without food and water (Cockram *et al.*, 1996). This would suggest that after a period of food deprivation, there is a maximum time that is spent feeding, which is not affected by the motion of a vehicle. The fall in plasma concentrations of free fatty acids as the sheep began to feed indicates a recovery from the period of deprivation.

Indicators of dehydration measured during this study all point to the onset of dehydration post-treatment, after the sheep began to feed. The increase in plasma sodium concentration during the first 12h post-treatment agrees with work by Blair-West & Brook (1969), who found a decrease in sodium excretion after feeding sheep dry food. Increases in plasma sodium concentrations are associated with the onset of drinking (Houpt, 1993), which may explain the higher water intakes over the first 12h post-treatment.

The increase in vasopressin seen post-treatment also occurred after the sheep began to feed. Increased plasma vasopressin concentration follows an increase in the osmotic concentration. Osmolality in sheep is normally maintained at 280-300 mosmol/kg (Tasker, 1980), all groups showed an increase in osmolality above 300 mosmol/kg as they began eating post-treatment. Previous work where sheep were deprived of water for 48h but continued to have access to food found that plasma osmolality increased (Parrott *et al.*, 1987; Parrott *et al.*, 1996). This may explain the changes in plasma vasopressin and osmolality seen over the first few hours with access to food in this study. The sheep spent most of this time feeding and may have drunk very little water during this period, showing that feeding can disturb the water balance of sheep.

3.4.1 Effect of a 24h food deprivation

Concentrations of plasma glucose decreased during treatment. This agrees with work by other authors (Lindsay & Leat, 1975; Graham & Phillips, 1981; Terashima *et al.*, 1982; Heitman *et al.*, 1986; Warriss *et al.*, 1987; 1989;) with an increase in the concentration of alternative energy fuels (beta-hydroxybutyrate, free fatty acids etc.) occurring at the same time. In the fed ruminant, over 90% of the glucose flux is due to gluconeogenesis (Bergman, 1973), with the production rate being directly correlated with the intake of digestible organic matter (Steel & Leng, 1973a), and up to 60% of glucose being derived from propionate produced in rumen fermentation (Steel & Leng, 1973b). This will partly explain the fall in plasma glucose concentration seen during fasting, as there is no addition to the digestible organic matter that is already present in the rumen at the start of the fasting period.

There was no significant change in plasma concentrations of beta-hydroxybutyrate over the period of food deprivation. This is in contrast with work by other authors, who found increased concentrations of plasma beta-hydroxybutyrate after 24h fasting (Warriss *et al.*, 1989; Knowles *et al.*, 1993). The concentrations of beta-hydroxybutyrate seen after 24h food deprivation in this study were lower than those seen by Warriss *et al.* (1989) (0·25mmol/l compared with

0·47mmol/l) and the pre-treatment levels were also lower (0·25mmol/l compared with 0·31mmol/l)

Concentrations of plasma free fatty acids increased during the treatment period. This is in agreement with other authors who have observed increases in plasma free fatty acid concentrations during both fasting and transport (Graham & Phillips, 1981; Terashima *et al.*, 1982; Heitman *et al.*, 1986; Warriss *et al.*, 1987, 1989, 1990; Knowles *et al.*, 1993). However, increases in plasma free fatty acid concentrations are also associated with a stress response and have been seen in response to handling procedures (Reid & Mills, 1962; Patterson, 1963; Purchas, 1973). In this study no increases in cortisol were observed during treatment, which would suggest that the increase in plasma free fatty acid concentration was a response to the food deprivation.

The lack of an effect of treatment on plasma cortisol concentration would suggest that confinement at temperatures of 14°C and 21°C for 24h without food and with/without water do not cause undue stress to the animals. However, the handlers were the same throughout the study, and animals were moved individually into climate chambers in a crate, and were therefore not exposed to loading, which is considered to be a critical point in relation to transport (Van Logtestijn & Romme, 1980). In addition, previous work has not found changes in plasma cortisol concentration during fasting (Reid & Mills, 1962; Warriss *et al.*, 1987; Wronska *et al.*, 1990), unless during the fasting period there was visual contact with feeding sheep (Wronska *et al.*, 1990).

The decrease in the proportion of scans spent ruminating during treatment agrees with other work on sheep confined in both stationary and moving vehicles (Cockram *et al.*, 1996), and the increase in the proportion of scans spent idling corresponds to the reduced proportion of scans spent ruminating during treatment. This study used a space allowance of 0·41m^2/sheep, which is higher than space allowances commonly used in the commercial transportation of sheep in Scotland (Jarvis & Cockram, 1994). As it has been suggested that rumination is inhibited in adverse situations (Beilharz, 1985), this decrease in rumination may indicate that the close confinement used in this study was aversive to the sheep.

In contrast with previous transport studies (Cockram *et al.*, 1996), the close confinement of sheep in a pen in this study had little effect on the standing and lying behaviour. All the sheep used in this study were blood-sampled. In the study by Cockram *et al.* (1996), the sheep used for behavioural observations were not blood sampled and would not have been disturbed by the blood sampling of other sheep pre-treatment, but may have been disturbed by blood sampling of other sheep while on the vehicles. This may have been a factor in the changes in standing and lying behaviour seen in that study.

There was an increase in the proportion of scans spent moving at the beginning of the 24h treatment, this was probably a response to the mixing of previously individually penned sheep. Cockram *et al.* (1996) observed little moving during 12h transport/confinement at a space allowance of $0{\cdot}22m^2$/sheep using lambs of similar liveweights to the sheep used in this study that had previously been group penned. This mixing and the higher space allowance used in this study enabling easier movement, may account for this difference.

The proportion of scans spent lying or standing was similar to that seen pre-treatment, but the provision of water reduced the proportion of scans spent standing and increased the proportion of scans spent lying. Although the water consumption during periods of food deprivation in this and other studies is lower than when sheep had access to food (Hecker *et al.*, 1964), this increased resting behaviour may indicate that it may have some impact on the comfort of the sheep. Those unable to consume this amount may experience increased dehydration or thirst and the motivation to find water may have increased.

The decrease in PCV (indicating an increase in the volume of plasma or a decrease in circulating red blood cells) during treatment agrees with observations of other authors (Warriss *et al.*, 1989; Knowles *et al.*, 1993). Previous work has found that during the first two days of food and water deprivation the rate of loss of fluid from rumen is at its greatest, resulting in an increase in plasma volume (Hecker *et al.*, 1964). This has been suggested as a method to keep the body's water balance unaltered for the first two days of a period of water deprivation, defending blood volume at the expense of both interstitial and intracellular fluid (Houpt, 1993). The

lack of an effect of temperature and provision of water in this experiment would suggest that the sheep may have been able to maintain plasma volume by using water from the rumen.

No effects of temperature and provision of water were found on the plasma osmolality during the treatment period. This agrees with previous work by Parrott *et al.* (1996) who found sheep could maintain normal plasma osmolality over a 48h deprivation at high ambient temperatures (35°C).

MacFarlane *et al.* (1961) found an effect of acclimatisation on the response of sheep to elevated temperatures, sheep that had not been acclimatised were found to be unable to tolerate water deprivation for as long as those that had been acclimatised to the environment. This could have implications for the export of sheep to Europe, particularly those transported to the South of Europe for slaughter where temperatures experienced may be considerably higher than any the sheep have previously experienced.

3.4.2 Post-treatment recovery

The initial lack of difference from pre-treatment levels for eating, ruminating, standing and lying probably resulted from the management practices. As the sheep were not fed *ad-libitum* hay, there was a high level of feeding when they were presented with new food, which corresponded to the time when the sheep were returned to their home pens post-treatment. There was however an increase in the amount of time spent feeding after the first hour compared with the equivalent time pre-treatment, which lasted for 3-4h. This and the lower proportion of scans being spent ruminating, suggests that there is an increased interest in food after a 24h period of food deprivation and that feeding is of a high priority after periods of food deprivation.

The increase in PCV seen immediately post-treatment will partly be caused by the increased salivary production that occurs during feeding. Stacy & Warner (1966) estimate that for every 1g dry food eaten, 2·7 ml saliva is swallowed. Work by Blair-West & Brook (1969) found that infusion of a solution resembling saliva during feeding prevented the fall in plasma volume seen without the infusion,

confirming that much of the initial fluid loss when feeding is as a result of increased salivary production. Christopherson & Webster (1972) also observed a decrease in plasma volume when eating, and found that 1·0-1·5l of fluid entered the gut from extracellular fluid space. This exceeds estimates of total salivary flow associated with ingestion of meals (Stacy & Warner, 1966) and suggests that large volumes of fluid pass directly across the rumen wall during eating (Ternouth, 1968).

3.5 Conclusions

The increase in feeding behaviour after 24h fasting lasted for 3-4h and was associated with a decrease in the plasma concentration of free fatty acids. This suggests a 3-4h lairage may be beneficial in enabling sheep to recover from a period of food deprivation.

However, the large intake of dry feed post-treatment caused signs of dehydration during the first 24h post-treatment. This post-treatment dehydration highlights a potential problem with the 1h feeding and watering interval required during longer journeys. Sheep have been observed to eat before they commence drinking, and if they do not have the opportunity to drink after feeding, this could increase the risk of dehydration during the continuation of any journey.

The provision of water and the temperature at which the sheep were held had little effect on the behaviour and physiology recorded in this study. The main factor seems to have been the period of confinement without food. There would not seem to be any obvious benefit in providing water during a journey as the water intake during the absence of food is low, and the volume of water drunk in this study was not sufficient to compensate for the increased requirement as the sheep begin to feed after treatment.

The temperatures used in this study were not as diverse as had been intended, and it would seem that close confinement of sheep at 21°C does not significantly increase the risk of dehydration or increase the effect of fasting above that seen at 14°C.

It would seem that provided sheep are given access to water when provided with food, and sufficient time is allowed to enable the sheep to feed and drink, being

held at 21°C does not cause any additional problems to the sheep in terms of their behaviour and physiology compared with 14°C.

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CHAPTER 4 Effect of providing lairage during a 24h journey on the behaviour, liveweight and food and water intake of sheep

Abstract

Changes in EU directives have reduced the maximum length of a journey before sheep are required to be watered, and if necessary fed, but allow journeys to continue after a 1h stoppage. An experiment was designed to investigate the effects of providing a period of lairage during a 24h road journey on the behavioural responses of sheep. Behavioural observations were made on lambs (liveweight 35kg) before, during and after either stationary confinement or transport for 24h with one of four types of lairage after 12h of the treatment (12 lairage, 3h lairage, 3h access to food and water on the vehicle, no lairage) (n=6).

During the lairage periods, sheep spent a greater proportion of scans eating (0·64) and a lower proportion of scans ruminating (0·15), than during the equivalent time of day pre-treatment (eating: 0·40; ruminating: 0·30). Sheep given food and water on the vehicles spent a lower proportion of scans eating (0·56) and lying (0·01) and a greater proportion of scans idling (0·38), than sheep that were unloaded and offered food and water (eating: 0·85; lying: 0·07; idling: 0·02). The median number of observed drinking bouts by sheep kept on the vehicles was lower (0) than for sheep that were unloaded and offered food and water (3·5).

During the second 12h treatment period sheep confined on a stationary vehicle after lairage spent a greater proportion of scans ruminating (0·39) than sheep that were transported after lairage (0·25). Sheep confined on stationary vehicles also spent a greater proportion of scans lying (0·66) and a lower proportion of scans

standing (0·33) than sheep that were transported after the lairage period (lying: 0·51; standing: 0·48).

After unloading, following the second 12h treatment period, sheep that had been given a 12h lairage period had the smallest change in liveweight (-0·83kg) during the treatment than those in the other treatment groups (3h lairage: -1·97kg; 3h vehicle: -2·21kg; no lairage: -2·5kg). All groups showed an increase in the proportion of scans spent eating post-treatment (0·52) compared with the equivalent time pre-treatment (0·44), the duration of the increase was shortest for sheep given a 12h lairage period.

The provision of a period of lairage facilitated post-treatment recovery, reducing the changes in liveweight during the treatment period and shortening the length of time for behaviour to return to pre-treatment levels. A 3h lairage period did not seem to allow time for sheep to feed and drink sufficiently to reduce the overall effect of the 24h journey. Providing food and water on the vehicles was not satisfactory, as less drinking was observed to take place compared with unloading the sheep into lairage.

4.1 Introduction

Large numbers of sheep are transported by road each year; approximately 16 million sheep are transported to slaughter in the UK (Warriss *et al.,* 1990), and live exports to Europe have been in excess of 1 million (MLC, 1996).

Legislation in both the UK and the EU has recognised public concerns about long distance transportation of animals, and requires the provision of a period of rest with food and water during long journeys. On long journeys, a period of lairage, where sheep are provided with the opportunity to eat, drink and rest, may minimise the effects of transportation and food and water deprivation. UK legislation until 1997 required sheep to be fed and watered every 15h unless the final destination could be reached within 16h (Welfare of Animals During Transport Order, 1994). EC directive 91/628 stipulated that on journeys exceeding 24h, animals should be rested, fed and watered and unloaded if necessary. A new EU directive 95/29/EC (European Council, 1995) that came into force in the UK in July 1997 (The Welfare

of Animals (Transport) Order, 1997), has reduced the maximum journey time to 14h (on approved vehicles), which may be followed by a further 14h journey if a 1h stoppage for watering (and feeding if necessary), is provided. However, little scientific work has been performed to assess the effect of providing a period for resting, feeding and watering during the transport on sheep.

Work by Cockram *et al.* (1996) showed that sheep could be transported for 12h without experiencing any major changes in biochemical measures of dehydration and fasting. A continuous 24h journey without food was found to result in an increase in the concentration of blood metabolites (plasma concentrations of free fatty acids, beta-hydroxybutyrate and urea), indicating the mobilisation of body energy reserves (Knowles *et al.*, 1995). After transporting sheep to Southern France from the UK on continuous journeys lasting 18h and 24h, there is some evidence of dehydration, with increases in the osmolality and the plasma concentrations of total protein and albumin (Knowles *et al.*, 1994). This evidence of a catabolic state, in conjunction with the increase in feeding behaviour seen post-transport (Cockram *et al.*, 1996), indicates that sheep may become hungry and thirsty during longer road journeys without access to food and water.

Transport causes a reduction in the amount of lying behaviour, and sheep confined on moving vehicles spend less time lying over the first few hours of a journey than sheep confined on stationary vehicles (Cockram *et al.*, 1996). This could result in sheep becoming fatigued, but studies have shown that after unloading sheep are primarily interested in feeding (Knowles *et al.*, 1994, 1995; Cockram *et al.*, 1996).

This experiment was performed in order to investigate the effect of providing a period of lairage during a 24h road journey by road on sheep. In particular, it examined the effect of the length of the lairage period, and whether the sheep were unloaded for the lairage period, on the post-treatment behaviour.

4.2 Material and methods

A 2x4 factorial design was used to investigate the effects of two transport treatments within which three types of lairage (during which sheep were provided with hay and water) were investigated.

4.2.1 Animals and management

The sheep used were 102 16-week-old Suffolk x Greyface lambs that were naive of the transportation process. The lambs had an average liveweight of 34·7 kg (s.e. 0·30).

Prior to the experiment, the lambs were kept on grass, where they had constant access to water, and were fed a commercial lamb creep feed containing monensin (0·3kg/sheep/day). One week prior to each treatment, 24 sheep were moved into the experimental unit, so that some acclimatisation could take place. There, they were randomly allocated into four groups of six, ensuring equal numbers of males and females in each group, and balancing for liveweight. All sheep were individually marked with a stockmarker (Super Sprayline Stockmarker, Ritchey Tagg Ltd.) to enable identification of each sheep during behavioural observations.

Two groups (used for behavioural observations) were group-housed on straw (pens 2·5m x 2·5m), and fed *ad-libitum* hay with continuous access to water. The remaining groups (used to obtain food and water intakes) were individually penned on shavings (pens 1·0m x 1·5m), and were offered known amounts of hay and water every 12h. The pens were cleaned between treatments and fresh bedding was provided as required. The shed was illuminated throughout the study, and was ventilated by natural ventilation through Yorkshire boards on one wall, and a ridge inlet in the roof.

4.2.2 Transporters

Two single deck, non-articulated Ford-Cargo six-cylinder diesel vehicles were used as sheep transporters. The confinement transporter was positioned outside of the building that housed the sheep throughout the study. The livestock area in each vehicle was divided into two pens (front pen: 0·8m x 2·22m, back pen: 1·6m x

2·22m), with chequerplate flooring covered with wood shavings. Ventilation on the stationary vehicle was achieved using an extractor fan, and the transport vehicle relied on natural ventilation. Observations were conducted from a separate observation area to cause minimal disturbance to the sheep. The pens were illuminated to allow observations at night.

4.2.3 Protocol

The transport treatments investigated were: confinement on a moving vehicle for 24h (transport), and confinement on a stationary vehicle for 24h (confinement). Three types of lairage during the 24h treatment and a continuous journey with no lairage period were investigated on successive weeks:

Table I. Description of lairage treatments and their experimental order.

Treatment	lairage type	treatment	week
A	12h lairage	12h treatment, 12h in home pen, 2nd 12h treatment	1
B	3h lairage	12h treatment, 3h in home pen, 2nd 12h treatment	2
C	3h lairage	12h treatment, 3h on vehicle, 2nd 12h treatment	3
D	no lairage	24h continuous treatment with no lairage	4

At the start of the treatment period, seven sheep were loaded into the front pen of each vehicle (one group individually penned sheep (n=6) + one additional non-experimental sheep), and 14 sheep were loaded into each back pen (one group of group-penned sheep (n=6), + 8 additional non-experimental sheep). This gave a space allowance of 0·25m^2/sheep on the vehicles.

On weeks 1, 2 and 4, two additional sheep were used that were not loaded onto the vehicles. These sheep were individually penned with access to known amounts of hay and water throughout the treatment period, acting as controls (n=6) for food and water intakes. The control group were spread over the four weeks of the experiment due to the limited space available in the building for housing the sheep.

On completion of the 24h treatment, sheep were returned to their home pens. The start of the treatments were arranged so that the second 12h treatment period and the 24h post-treatment period began at the same time of day each week.

4.2.4 Liveweight

The liveweights of the individually penned sheep were measured using a weigh-crate: 12h before and immediately before the start of the treatment period, at the end of the treatment period and 48h post-treatment.

4.2.5 Hay and water intakes

The hay and water intakes of the individually penned sheep were recorded for two consecutive 12h periods before treatment, during the treatment period and for two consecutive 12h periods post-treatment. Known amounts of hay and water were offered every 12h and refusals were measured at the end of each 12h period. For sheep fed on the vehicles, two hay racks and two water buckets containing known amounts of hay and water were placed in each pen during the 3h period when the transport vehicle was stationary.

4.2.6 Behavioural observations

Behaviour of the sheep in the group pens was recorded by direct observation from an elevated platform before and after treatment and during lairage periods.

Observations were recorded by scan sampling each sheep every 6 minutes for 24h pre-treatment, throughout the treatment and lairage periods and for the first 24h post-treatment. The behaviours recorded by scan sampling were:

standing (upright stationary posture)

moving (upright posture involving change in location)

lying (recumbent posture with body in contact with floor)

eating (ingestion of food followed by jaw movements and swallowing)

ruminating (regurgitation, jaw movements and swallowing that is not preceded by eating)

investigating (sniffing and licking environment)

idling (performing no apparent behaviour)

panting (rapid breathing with mouth open).

other (other behaviour not already described)

In addition, the incidence of drinking bouts (muzzle immersed in water for >5 seconds) was recorded continuously for each sheep in the group pens.

Observations for each sheep were recorded on hand-held computers (PSION LZ64), and analysed using behavioural software (Noldus Information Technology, 1993; 1994).

4.2.7 Statistical analysis

The statistical analysis of data generally requires the independence of the experimental units involved. This would require either the individual penning of sheep or an increase in the number of groups used in this experiment. Sheep are social animals, with high levels of synchronisity in their behaviour, for example, social facilitation has been shown to initiate feeding (Rook & Penning, 1991). This synchronisity may still occur when sheep are individually penned, the sight of a hungry sheep feeding has been shown to stimulate satiated sheep to resume eating (Tribe, 1950; Rook & Penning, 1991). This suggests that the behaviour of individually penned sheep may not be truly independent. The only method of ensuring the independence of the behaviour in individually penned sheep is to house them in isolation from other sheep. This has been shown to be both stressful, causing increases in cortisol levels (Parrott *et al.,* 1987) and heart rate (Syme & Elphick, 1982). Isolation has also been shown to be aversive, with sheep taking longer to run a race (when they were isolated at the end of it) compared with being loaded onto a vehicle or transported (Douglas-Hudson, 1992) and preferring human presence (in the presence of other sheep) to isolation in a choice test (Rushen, 1986). Placing a mirror in the pen with an isolated sheep was found to reduce the magnitude of the response to isolation (Parrott *et al.,* 1988), but there was still a response and the authors suggested that the sheep treated the reflection as an unfamiliar animal.

In addition, individual penning has been shown to adversely affect the behaviour of sheep (Marsden & Wood-Gush, 1986) which would reduce the benefit of applying any findings to the commercial situation, as sheep are housed and transported in groups and their behaviour will be influenced by conspecifics. This experiment is particularly interested in the effect of the food deprivation associated

with transport and the post-treatment recovery and so any influence of the sheep on the feeding behaviour of others is important as this will occur during commercial transportation.

Some sheep in this experiment were individually penned, these sheep were blood sampled as part of the larger project that this reported experiment was part of. These sheep were also used to obtain information on the food and water intakes of the sheep during the experiment. While the data obtained was more suitable for statistical analysis, work has shown that sheep housed individually (and also when isolated) consume less food than those housed in groups (Webster *et al.*, 1972). This means that any results obtained are likely to underestimate the intakes of sheep of a similar size in the commercial situation. This reduction in feeding and the potential adverse effects on behaviour make it preferable to use group-housed sheep when recording behaviour to ensure that (as far as possible in an experimental situation) the sheep behave as they would under commercial conditions. To do this would require the use of an increased number of sheep to increase the number of replicates in each treatment. This was not financially feasible during this project. However, an increasing number of experiments on sheep transport have been published, which allows us to compare the consistency of the results in terms of the effect of transport and the associated food deprivation.

As a result of the non-independence of the data from the behavioural observations, the majority of the results from the analysis will be reported descriptively, some significant effects will be reported. In both cases, any consistency with published work will be commented on.

For each sheep in the group pens, the proportion of scans spent in each behaviour was calculated for every hour of the observation periods. These proportions were used for the statistical analyses (n=6). A repeated measures analysis of variance (Laird & Ware, 1982) using the mixed procedure within SAS version 6 (SAS Institute Inc. Cary USA) was used to examine the effects of treatment (confinement and transport), lairage (12h, 3h, 3h on vehicle and no lairage) and time on the proportion of scans spent performing each behaviour. Where there were interactions between treatment, lairage and time the differences

between least-square means were examined. Analyses were performed to make specific hourly comparisons during equivalent times of day for: the 3h period during treatment when sheep were lairaged or fed and watered on the vehicle; the three lairage periods and the equivalent times pre-treatment; the second 12h treatment period and the equivalent time pre-treatment; the first 12h post-treatment and the equivalent time pre-treatment and the second 12h post-treatment and the equivalent time pre-treatment.

This analysis was repeated for the hay and water intakes of the individually penned sheep (n=6), performing separate analyses of variance for each 12h period. Liveweights were analysed using a two-way ANCOVA (transport and lairage) using the pre-treatment liveweights as the covariate, and by using a paired t-test (time) (n=6). Where there were significant effects, the significant differences were found by calculating the least significant differences (Snedecor & Cochran, 1980). The changes in liveweight were analysed using a two-way ANOVA to examine the effects of transport and lairage treatments (n=6).

For the group penned sheep, the total number of drinking bouts performed by each sheep during each 12h period during the behavioural observations was calculated. The effects of lairage and transport treatments on these values were analysed by using Kruskal-Wallis tests (n=6). Where significant effects of lairage were found, multiple comparisons were made between each pair of treatments, comparing the difference in ranks to the critical difference (Siegel & Castellan, 1988). The effect of time was analysed by using Wilcoxon signed ranks test (n=6).

4.3 Results

4.3.1 Liveweight

There were significant effects of transport and lairage on the mean liveweight after the second 12h treatment period (Table II), but there was no effect of the transport*lairage interaction. The mean liveweight of control sheep was greater than of those that had been confined on moving or stationary vehicles.

Table II. Effects of treatment and lairage on the adjusted mean liveweights (s.e.) at unloading after the second 12h treatment period (n=6).

		Transport	Confinement	Control	
Liveweight (kg)					
		32·6[a]	32·9[a]	35·2[b]	
		(0·55)	(0·59)	(1·03)	
	12h lairage	3h lairage	3h vehicle	no lairage	control
Liveweight (kg)					
	33·7[a]	32·8[a]	32·2[b]	32·3[a]	35·2[c]
	(0·55)	(1·12)	(0·82)	(0·44)	(1·03)

a,b,c. Values with different superscripts in the same row are significantly different, $p<0·05$.

Control sheep had a greater mean liveweight than the four lairage groups after the second 12h treatment period ($p<0·05$). The groups that had been given food and water on the vehicle had a lower mean liveweight than the other three lairage groups ($p<0.05$). Control sheep showed no change in liveweight between pre-treatment and the time that unloading occurred in the other groups. All other groups showed weight loss, with the sheep that had been given 12h lairage having the smallest change in liveweight (mean change: 12h lairage -0·83kg s.e. 0·19; 3h lairage -1·97kg s.e. 0·27; 3h vehicle -2·21kg s.e. 0·24; no lairage -2·5kg s.e. 0·23, $p<0·001$)

The mean liveweights at the end of the treatment period were lower than pre-treatment (pre-treatment: 34·7kg s.e. 0·30; unloading: 33·1kg s.e. 0·40, $p<0·0001$), but after 48h the mean liveweights had returned to pre-treatment level (48h post-treatment: 34·5 kg s.e. 0·41).

4.3.2 Hay and water intake

During lairage

Table III shows the hay and water intakes of individually penned sheep during and after treatment for each lairage treatment.

Table III. Mean hay and water intakes (s.e.) of individually penned sheep for each 12h period over the whole treatment period (n=6).

	Pre-treatment 1	Pre-treatment 2	Lairage	Post-treatment 1	Post-treatment 2
Hay Intake (kg)					
12h lairage	0·43 (0·04)	0·42 (0·04)	$0·83^{a}$ (0·04)	$0·76^{b}$ (0·04)	0·42 (0·04)
3h lairage	0·83 (0·04)	0·51 (0·04)	$0·47^{b}$ (0·04)	$0·69^{b}$ (0·04)	0·50 (0·04)
3h vehicle	0·53 (0·04)	0·50 (0·04)		$0·65^{b}$ (0·04)	0·52 (0·04)
No lairage	0·92 (0·04)	0·46 (0·04)		$0·89^{a}$ (0·04)	0·46 (0·04)
Control	0·71 (0·06)	0·43 (0·06)	$0·71^{a}$ (0·06)	$0·62^{b}$ (0·06)	0·49 (0·06)
Water Intake (litres)					
12h lairage	1·4 (0·19)	1·4 (0·19)	$1·6^{a}$ (0·19)	$1·7^{b}$ (0·19)	1·1 (0·19)
3h lairage	$1·7^{x}$ (0·19)	1·1 (0·19)	$0·5^{yb}$ (0·19)	$2·1^{abz}$ (0·19)	1·1 (0·19)
3h vehicle	$0·8^{x}$ (0·19)	$0·7^{x}$ (0·19)		$2·5^{ay}$ (0·19)	$1·5^{az}$ (0·19)
No lairage	1·4 (0·19)	1·8 (0·19)		$1·8^{b}$ (0·19)	$0·6^{b}$ (0·19)
Control	1·1 (0·27)	2·0 (0·27)	$1·3^{a}$ (0·27)	$0·8^{c}$ (0·27)	1·2 (0·27)

a,b,c. Values with different superscripts in the same column are significantly different, $p<0·05$.
x,y,z Values with different superscripts in the same row are significantly different, $p<0·01$.

Sheep unloaded for a 3h lairage period drank less water than at the equivalent time pre-treatment. Control sheep and sheep given a 12h lairage period ate more hay ($p<0·0001$) and drank more water ($p<0·0001$), during the lairage period than those given a 3h lairage period off the vehicle.

The mean hay intakes during lairage for sheep kept on the vehicles were: stationary vehicle: front pen-0·43 kg, back pen-0·49 kg; moving vehicle: front pen-0·38 kg, back pen-0·35 kg. The mean water intakes during lairage for sheep kept on the vehicles were: stationary vehicle: front pen-0·7 litres, back pen-0·4 litres; moving vehicle- front pen-0·5 litres, back pen- 0·5 litres.

Over the first 3h of the lairage period, sheep that were fed and watered on the vehicles had a lower number of drinking bouts than those that were unloaded

(median (Q1, Q3): 12h lairage 5 (1·75, 5); 3h lairage 3.5 (1·75, 7); 3h vehicle 0 (0, 1)).

Post-treatment

During the first 12h post-treatment, those that had not been given a lairage period ate more hay than the other groups ($p<0·05$). There were no differences between lairage treatments during the second 12h period post-treatment.

During the first 12h post-treatment, those that had been given a 12h lairage period drank less water than those that had been given food and water on the vehicle ($p<0·01$). Control sheep drank less water during the first 12h post-treatment than the four lairage treatments ($p<0·0001$). During the first 24h post-treatment, those that had been given food and water on the vehicle drank more than those that had not been given a lairage period ($p<0·01$).

Sheep that had been confined in a stationary vehicle drank more during the first 12h post-treatment (2·2 litres s.e. 0·15) than at the equivalent time pre-treatment (1·1 litres s.e. 0·15, $p<0·0001$). Sheep given food and water on the vehicle drank more water post-treatment than at the equivalent time pre-treatment (Table III, $p<0·01$).

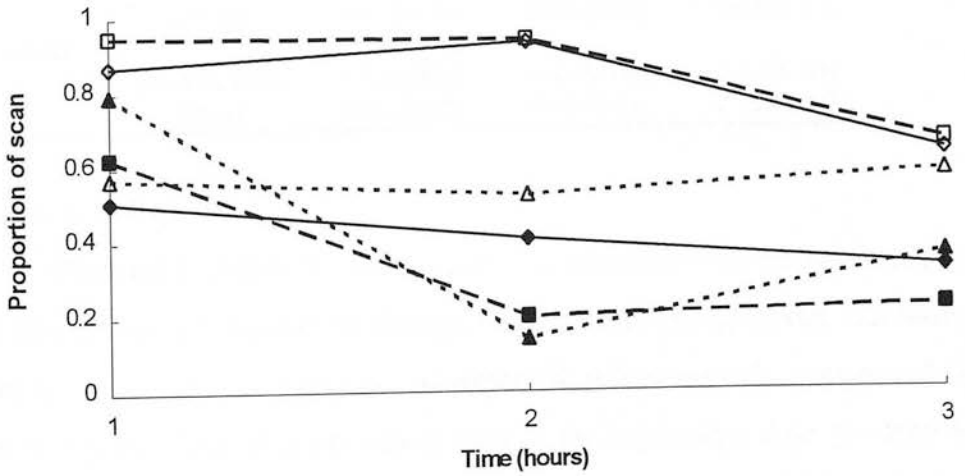

Figure 4.1. Effect of three lairage treatments on the mean proportion of scans spent eating during the first 3h of lairage (open symbols) compared with the equivalent time pre-treatment (closed symbols) (n=6). ◆- A (12h lairage off vehicle); ■- B (3h lairage off vehicle); ▲ - C (3h lairage on the vehicle).

4.3.3 Behaviour

During lairage

Table IV shows the mean proportion of scans spent performing each behaviour during the lairage period. Although only a small number of groups were studied, there were strong indications that the type of lairage provided affected behaviour.

Table IV. Mean proportion of scans (s.e.) spent in each behaviour during the lairage period and the equivalent time pre-treatment (n=6).

		12h lairage	3h lairage	3h on vehicle
eat				
	pre-treatment	0·46 (0·02)	0·35 (0·04)	0·40 (0·04)
	lairage	0·51 (0·03)	0·85 (0·04)	0·56 (0·05)
idle				
	pre-treatment	0·05 (0·01)	0·03 (0·01)	0·04 (0·01)
	lairage	0·12 (0·02)	0.02 (0·01)	0·38 (0·06)
investigate				
	pre-treatment	0·03 (0·005)	0·005 (0·004)	0·02 (0·007)
	lairage	0·006 (0·003)	0·009 (0·005)	0·008 (0·004)
lie				
	pre-treatment	0·44 (0·02)	0·58 (0·04)	0·52 (0·04)
	lairage	0·43 (0·02)	0·07 (0·02)	0·01 (0·01)
move				
	pre-treatment	0·02 (0·004)	0·01 (0·005)	0·01 (0·005)
	lairage	0·007 (0·002)	0·009 (0·005)	0·003 (0·003)
other				
	pre-treatment	0·005 (0·001)	0·01 (0·004)	0·01 (0·006)
	lairage	0·008 (0·003)	0·02 (0·009)	0
ruminate				
	pre-treatment	0·23 (0·01)	0·38 (0·03)	0·29 (0·04)
	lairage	0·33 (0·02)	0·09 (0·03)	0·04 (0·01)
stand				
	pre-treatment	0·54 (0·02)	0·41 (0·04)	0·47 (0·04)
	lairage	0·56 (0·02)	0·92 (0·03)	0·99 (0·01)

Feeding behaviour

Figure 4.1 shows the mean proportion of scans spent eating during the first 3h of lairage and the equivalent time pre-treatment for sheep given 12h lairage and 3h lairage on and off the vehicles. As expected, following 12h treatment sheep spent a greater proportion of scans eating than at the equivalent time pre-treatment. For sheep given access to food and water for 3h either on and off the vehicles this was seen during the whole 3h (Table IV). For sheep given a 12h lairage period this difference was seen during the first 3h of the lairage period (pre-treatment: 0·42 s.e.

0·05; lairage: 0·81 s.e. 0·03). Sheep that were kept on the vehicles spent a lower proportion of scans eating than the groups that were unloaded during the first 3h of lairage (12h lairage: 0·81 s.e. 0·03; 3h lairage: 0·85 s.e. 0·04; 3h vehicle: 0·56 s.e. 0·05) (n=6 sheep per group, $p<0·0001$, but results from individual sheep were not fully independent). Sheep that had been confined on stationary vehicles spent a greater proportion of scans eating than those that had been confined on moving vehicles (confined: 0·93 s.e. 0·02; transported 0·78 s.e. 0·06).

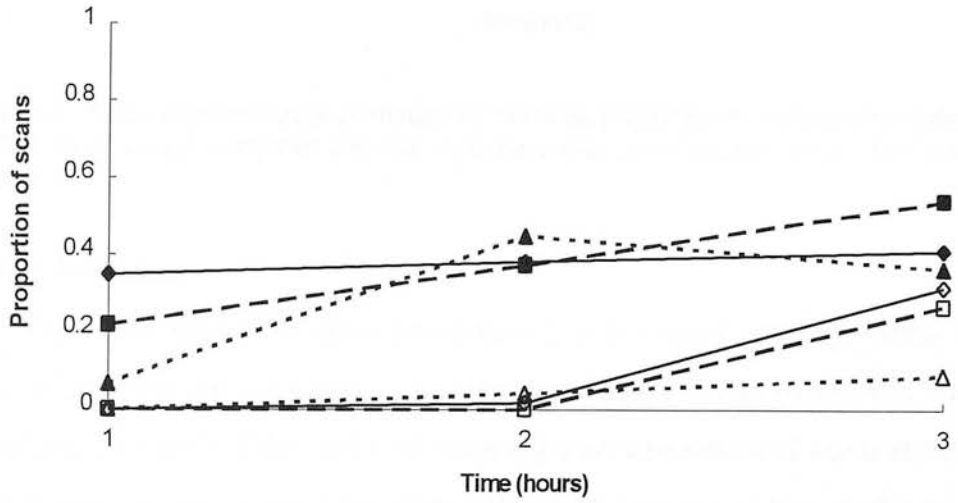

Figure 4.2. Effect of three lairage treatments on the mean proportion of scans spent ruminating during the first 3h of lairage compared with the equivalent time pre-treatment (n=6). For legends see Figure 4.1.

Figure 4.2 shows the mean proportion of scans spent ruminating during the first 3h of lairage and the equivalent times pre-treatment for the groups given access to food and water.

Sheep given a 3h lairage period on or off the vehicle spent a lower proportion of scans ruminating than during the equivalent time pre-treatment (Table IV). This difference was also seen for sheep given a 12h lairage, but only during the first 2h of the lairage period (pre-treatment: 0·37 s.e. 0·06; lairage: 0·01 s.e. 0·01). Sheep that remained on the vehicle spent a lower proportion of scans ruminating during lairage (0·04 s.e. 0·01) than those that were unloaded (3h lairage 0·09 s.e. 0·03).

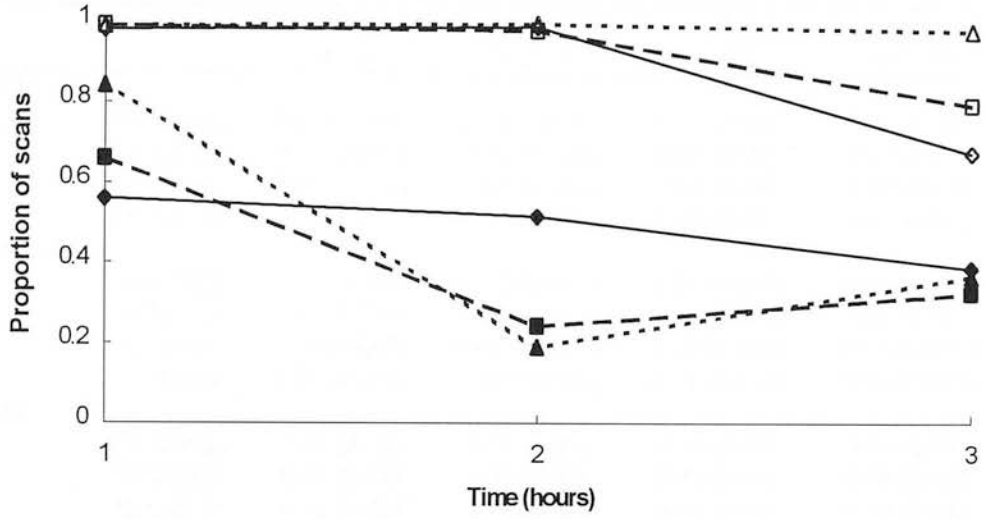

Figure 4.3. Effect of three lairage treatments on the mean proportion of scans spent standing during the first 3h of lairage compared with the equivalent time pre-treatment (n=6). For legends, see Figure 4.1.

Resting behaviour

Figure 4.3 shows the mean proportion of scans spent standing during the first 3h of lairage and the equivalent time pre-treatment. The groups given a 3h lairage period (both on and off the vehicles) spent a greater proportion of scans standing and a lower proportion of scans lying during the 3h lairage period than at the equivalent time pre-treatment (Table IV). Sheep given a 12h lairage period spent a greater proportion of scans standing than at the equivalent time pre-treatment during the first 4h of the lairage period (pre-treatment: 0·52 s.e. 0·04; lairage: 0·76 s.e. 0·03). During the first 3h of lairage, these sheep spent a lower proportion of scans lying than at the equivalent time pre-treatment (pre-treatment: 0·51 s.e. 0·05; lairage: 0·10 s.e. 0·03)..

During the first 2h of lairage there was no difference in the proportion of scans spent standing, lying or moving by the groups given a period of lairage. During the third hour of lairage, sheep that had been given food and water on the vehicles spent a lower proportion of scans lying (0·01 s.e. 0·01), and a greater proportion of scans standing (0·98 s.e. 0·02), than those that were unloaded for 3h (lying: 0·20 s.e. 0·07; standing: 0·80 s.e. 0·07) or 12h (lying: 0·29 s.e. 0·08; standing: 0·68 s.e. 0·08).

Table V. Mean proportion of scans (s.e.) spent in each behaviour during the 24h treatment period and the equivalent time pre-treatment (n=6).

		Pre treatment		During treatment	
		1st 12h	2nd 12h	1st 12h	2nd 12h
idle					
	12h lairage	0·05 (0·008)	0·02 (0·005)	0·73 (0·02)	0·28 (0·04)
	3h lairage	0·04 (0·005)	0·03 (0·005)	0·85 (0·02)	0·25 (0·04)
	3h vehicle	0·03 (0·004)	0·02 (0·003)	0·83 (0·03)	0·20 (0·02)
	no lairage	0·04 (0·01)	0·01 (0·006)	0·88 (0·03)	0·32 (0·04)
investigate					
	12h lairage	0·03 (0·005)	0·02 (0·002)	0·04 (0·007)	0·01 (0·003)
	3h lairage	0·01 (0·003)	0·01 (0·002)	0·03 (0·006)	0·02 (0·005)
	3h vehicle	0·02 (0·003)	0·007 (0·002)	0·02 (0·004)	0·02 (0·003)
	no lairage	0·01 (0·004)	0·007 (0·003)	0·01 (0·004)	0·02 (0·006)
lie					
	12h lairage	0·44 (0·02)	0·76 (0·02)	0·45 (0·05)	0·54 (0·05)
	3h lairage	0·47 (0·02)	0·71 (0·02)	0·44 (0·04)	0·56 (0·05)
	3h vehicle	0·51 (0·02)	0·74 (0·02)	0·53 (0·05)	0·65 (0·03)
	no lairage	0·49 (0·02)	0·77 (0·02)	0·45 (0·05)	0·60 (0·04)
move					
	12h lairage	0·02 (0·004)	0·02 (0·003)	0·009 (0·003)	0·01 (0·003)
	3h lairage	0·009 (0·003)	0·01 (0·003)	0·004 (0·002)	0·002 (0·001)
	3h vehicle	0·01 (0·002)	0·008 (0·003)	0·004 (0·002)	0·006 (0·002)
	no lairage	0·01 (0·003)	0·01 (0·003)	0·004 (0·002)	0·002 (0·001)
other					
	12h lairage	0·005 (0·001)	0·009 (0·001)	0	0·001 (0·001)
	3h lairage	0·009 (0·004)	0·006 (0·002)	0·007 (0·004)	0·003 (0·002)
	3h vehicle	0·006 (0·002)	0·002 (0·001)	0·006 (0·001)	0·003 (0·001)
	no lairage	0·002 (0·002)	0·001 (0·001)	0·006 (0·003)	0·004 (0·002)
ruminate					
	12h lairage	0·23 (0·01)	0·40 (0·02)	0·24 (0·02)	0·37 (0·03)
	3h lairage	0·31 (0·02)	0·49 (0·02)	0·13 (0·02)	0·40 (0·02)
	3h vehicle	0·35 (0·01)	0·49 (0·01)	0·14 (0·03)	0·37 (0·03)
	no lairage	0·28 (0·02)	0·46 (0·01)	0·11 (0·03)	0·15 (0·02)
stand					
	12h lairage	0·54 (0·03)	0·22 (0·02)	0·54 (0·05)	0·45 (0·05)
	3h lairage	0·52 (0·02)	0·28 (0·02)	0·56 (0·04)	0·43 (0·05)
	3h vehicle	0·48 (0·02)	0·25 (0·02)	0·51 (0·05)	0·34 (0·03)
	no lairage	0·50 (0·02)	0·21 (0·02)	0·60 (0·05)	0·40 (0·04)

Other behaviour

No panting was observed during any observation period. Sheep that were given food and water on the vehicles spent a greater proportion of scans idling than at the equivalent time pre-treatment for the whole 3h lairage period (Table IV). For the group given 12h lairage, a greater proportion of scans were spent idling than at the equivalent time pre-treatment from 8-12h from the start of the lairage period (pre-treatment: 0·04 s.e. 0·01; lairage: 0·19 s.e. 0·02). The sheep that were unloaded

for 3h and 12h spent a lower proportion of scans idling than those that were given food and water on the vehicles during the lairage period (Table IV).

During second 12h treatment period

Table V shows the mean proportion of scans spent in each behaviour during treatment and the equivalent times pre-treatment.

Feeding behaviour

For sheep that had been given food and water, those that had been confined on a moving vehicle spent a lower proportion of scans ruminating than those that had been confined on a stationary vehicle (confined: 0·47 s.e. 0·01; transported 0·29 s.e. 0·01). This was seen during the first 6h of the second 12h treatment period for sheep unloaded for 12h or fed on the vehicles, but differences were seen for most of the 2nd 12h treatment period for sheep that had been unloaded for 3h.

As expected. all sheep showed a decrease in the proportion of scans spent ruminating compared with the equivalent time pre-treatment (Table V).

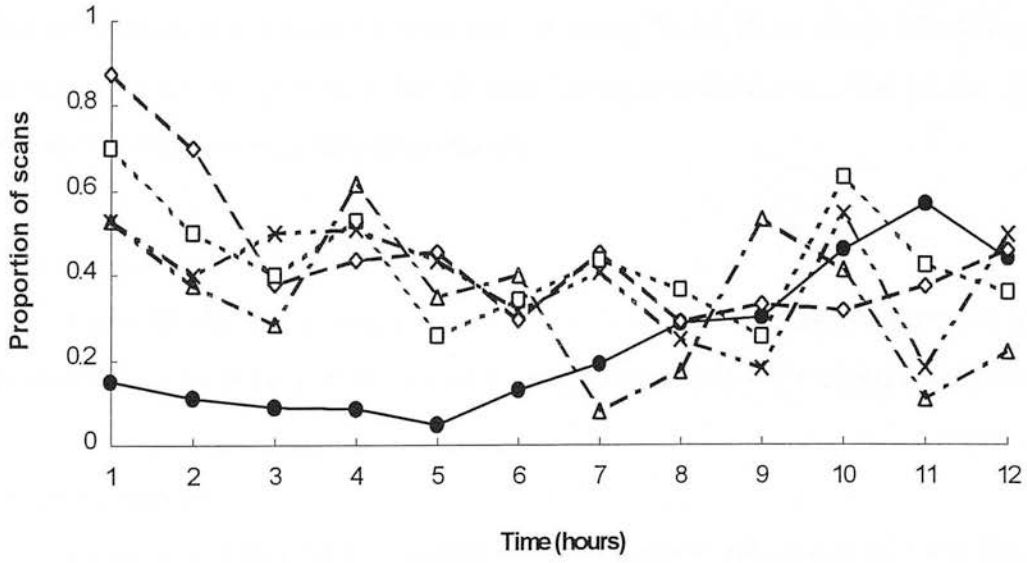

Figure 4.4. Effect of four lairage treatments on the mean proportion of scans spent standing during the second 12h treatment period and the equivalent time pre-treatment (n=6). ●- pre-treatment (averaged over the four treatments); ◊- A (12h lairage off the vehicle); □- B (3h lairage off the vehicle); Δ- C (3h lairage on the vehicle); X- D (no lairage).

Resting behaviour

At the start of the second 12h treatment period, sheep that had been given a 12h lairage period spent a greater proportion of scans standing (0·79 s.e. 0·06) than those from the other lairage treatments (3h lairage: 0·60 s.e. 0·07; 3h vehicle: 0·45 s.e. 0·07; no lairage: 0·47 s.e. 0·09) (Figure 4.4).

During the first 5h of the second 12h treatment period, sheep confined on a stationary vehicle spent a lower proportion of scans standing (0·37 s.e. 0·04), and a greater proportion of scans lying (0·63 s.e. 0·04), than those confined on a moving vehicle (standing: 0·61 s.e. 0·03; lying: 0·38 s.e. 0·03). Sheep confined on a moving vehicle spent a greater proportion of scans standing (0·56 s.e. 0·03), and a lower proportion of scans lying (0·43 s.e. 0·03) than at the equivalent time pre-treatment (standing: 0·11 s.e. 0·02; lying: 0·88 s.e. 0·02) for the first 7h of the second 12h treatment period. For sheep confined on a stationary vehicle this difference was seen for the first 5h of the second 12h treatment period (standing: pre-treatment: 0·10 s.e. 0·02; during treatment: 0·37 s.e. 0·02; lying: pre-treatment: 0·88 s.e. 0·04; during treatment: 0·63).

This increase in the proportion of scans spent standing and decrease in the proportion of scans spent lying compared with the equivalent time pre-treatment was also seen across the 4 lairage treatments. It lasted 7h for those given 12h lairage, 6h for those not given a period of lairage and those given food and water on the vehicle and 4h for those given a 3h lairage period.

Post-treatment

Table VI shows the mean proportion of scans spent in each behaviour during the two 12h periods post-treatment and the equivalent 12h periods pre-treatment.

Feeding behaviour

Figure 4.5 shows the mean proportion of scans spent eating over the first 24h post-treatment and the equivalent time pre-treatment for the four lairage treatments. During the first hour post-treatment, there was no effect of lairage treatment on the proportion of scans spent eating (12h lairage: 0·88 s.e. 0·02; 3h lairage: 0·96 s.e.

0·02; 3h vehicle: 0·97 s.e. 0·02; no lairage: 0·98 s.e. 0·02). From 2-5h post-treatment sheep that had not been given a lairage period spent a greater proportion of scans eating than sheep from the other three lairage treatments (12h lairage: 0·63 s.e. 0·04; 3h lairage: 0·71 s.e. 0·04; 3h vehicle: 0·63 s.e. 0·03; no lairage 0·81 s.e. 0·03) (n=6 sheep per group, $p<0·0001$, but the results from the individual sheep were not fully independent).

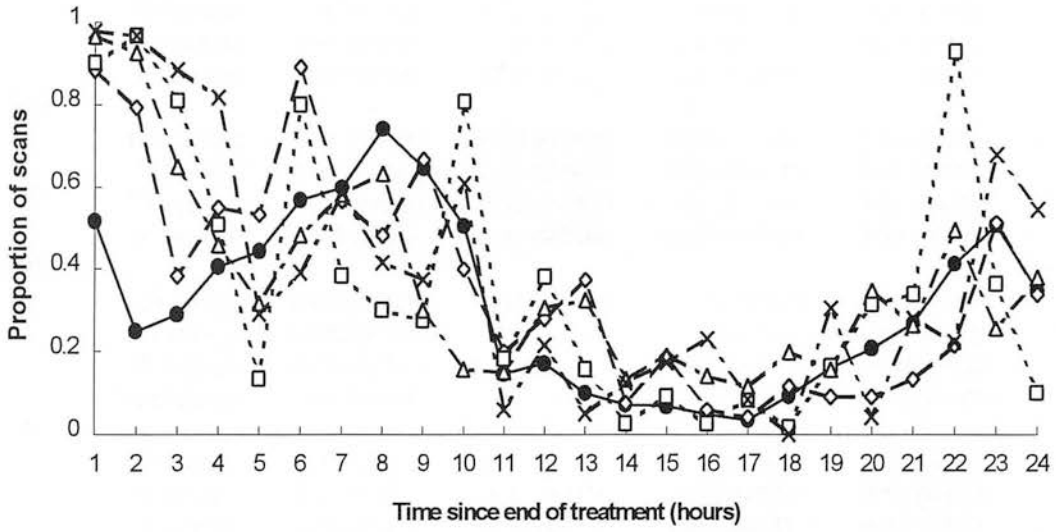

Figure 4.5. Effect of four lairage treatments on the mean proportion of scans spent eating post-treatment compared with the equivalent time pre-treatment (n=6). For legends, see Figure 4.4.

There was no effect of lairage treatment on the proportion of scans spent ruminating for the first 2h post-treatment (Figure 4.6). From 5-7h post-treatment, the group that had been given a 12h lairage period spent a lower proportion of scans ruminating than the other groups (12h lairage: 0·23 s.e. 0·03; 3h lairage: 0·42 s.e. 0·04; 3h vehicle: 0·38 s.e. 0·02; no lairage: 0·48 s.e. 0·03).

As expected, there was an increase in the proportion of scans spent eating post-treatment compared with the equivalent time pre-treatment (Figure 4.5, n=6 sheep per group, $p<0·0001$, but the results from the individual sheep were not fully independent). This increase lasted longer for the groups given either a 3h lairage period off the vehicle or no lairage period (4h), than for the groups given 3h lairage period on the vehicle (3h) or those given a 12h lairage period (2h), after which sheep

returned to pre-treatment levels. There was a corresponding decrease in the proportion of scans spent ruminating compared with the equivalent time pre-treatment(Figure 4.6), lasting for similar times as the increase in feeding.

Table VI. Mean proportion of scans (s.e.) spent in each behaviour during the 24h post-treatment period and the equivalent time pre-treatment (n=6).

		Pre treatment		Post treatment	
		1st 12h	2nd 12h	1st 12h	2nd 12h
eat					
	12h lairage	0·46 (0·02)	0·17 (0·02)	0·55 (0·02)	0·19 (0·02)
	3h lairage	0·45 (0·02)	0·23 (0·02)	0·54 (0·03)	0·22 (0·02)
	3h vehicle	0·42 (0·02)	0·21 (0·02)	0·49 (0·02)	0·25 (0·02)
	no lairage	0·44 (0·03)	0·17 (0·02)	0·55 (0·02)	0·23 (0·02)
idle					
	12h lairage	0·05 (0·008)	0·02 (0·005)	0·02 (0·004)	0·03 (0·005)
	3h lairage	0·04 (0·005)	0·03 (0·005)	0·02 (0·004)	0·02 (0·004)
	3h vehicle	0·03 (0·004)	0·02 (0·003)	0·02 (0·006)	0·01 (0·005)
	no lairage	0·04 (0·01)	0·01 (0·006)	0·02 (0·004)	0·01 (0·003)
investigate					
	12h lairage	0·03 (0·005)	0·02 (0·002)	0·006 (0·002)	0·01 (0·003)
	3h lairage	0·008 (0·003)	0·01 (0·002)	0·02 (0·006)	0·007 (0·002)
	3h vehicle	0·02 (0·003)	0·007 (0·002)	0·01 (0·003)	0·006 (0·01)
	no lairage	0·01 (0·004)	0·007 (0·003)	0·02 (0·004)	0·007 (0·003)
lie					
	12h lairage	0·44 (0·02)	0·76 (0·02)	0·40 (0·02)	0·75 (0·02)
	3h lairage	0·47 (0·02)	0·71 (0·02)	0·38 (0·02)	0·73 (0·02)
	3h vehicle	0·51 (0·02)	0·74 (0·02)	0·45 (0·01)	0·72 (0·02)
	no lairage	0·49 (0·02)	0·77 (0·02)	0·39 (0·02)	0·74 (0·02)
move					
	12h lairage	0·02 (0·004)	0·02 (0·003)	0·02 (0·003)	0·01 (0·003)
	3h lairage	0·009 (0·003)	0·01 (0·003)	0·01 (0·003)	0·01 (0·003)
	3h vehicle	0·01 (0·002)	0·008 (0·003)	0.008 (0·002)	0·005 (0·001)
	no lairage	0·01 (0·003)	0·01 (0·003)	0·007 (0·003)	0·01 (0·003)
other					
	12h lairage	0·005 (0·001)	0·009 (0·001)	0·004 (0·002)	0·006 (0·001)
	3h lairage	0·009 (0·004)	0·006 (0·002)	0·003 (0·002)	0·004 (0·001)
	3h vehicle	0·006 (0·002)	0·002 (0·001)	0·005 (0·001)	0·001 (0·001)
	no lairage	0·002 (0·002)	0·001 (0·001)	0·005 (0·002)	0·003 (0·002)
ruminate					
	12h lairage	0·23 (0·01)	0·40 (0·02)	0·29 (0·02)	0·51 (0·02)
	3h lairage	0·31 (0·02)	0·49 (0·02)	0·31 (0·02)	0·46 (0·02)
	3h vehicle	0·35 (0·01)	0·49 (0·01)	0·30 (0·02)	0·43 (0·02)
	no lairage	0·28 (0·02)	0·46 (0·01)	0·30 (0·02)	0·48 (0·01)
stand					
	12h lairage	0·54 (0·03)	0·22 (0·02)	0·58 (0·02)	0·24 (0·02)
	3h lairage	0·52 (0·02)	0·28 (0·02)	0·61 (0·02)	0·26 (0·02)
	3h vehicle	0·48 (0·02)	0·25 (0·02)	0·54 (0·02)	0·28 (0·02)
	no lairage	0·50 (0·02)	0·21 (0·02)	0·61 (0·02)	0·25 (0·02)

From 3-9h post-treatment, sheep that had been confined on a stationary vehicle spent a greater proportion of scans ruminating compared with the equivalent time pre-treatment (pre-treatment: 0·23 s.e. 0·01; post-treatment: 0·33 s.e. 0·01). For sheep that had been confined on a moving vehicle, this increase lasted until 11h post-treatment (pre-treatment: 0·25 s.e. 0·02; post-treatment: 0·37 s.e. 0·02).

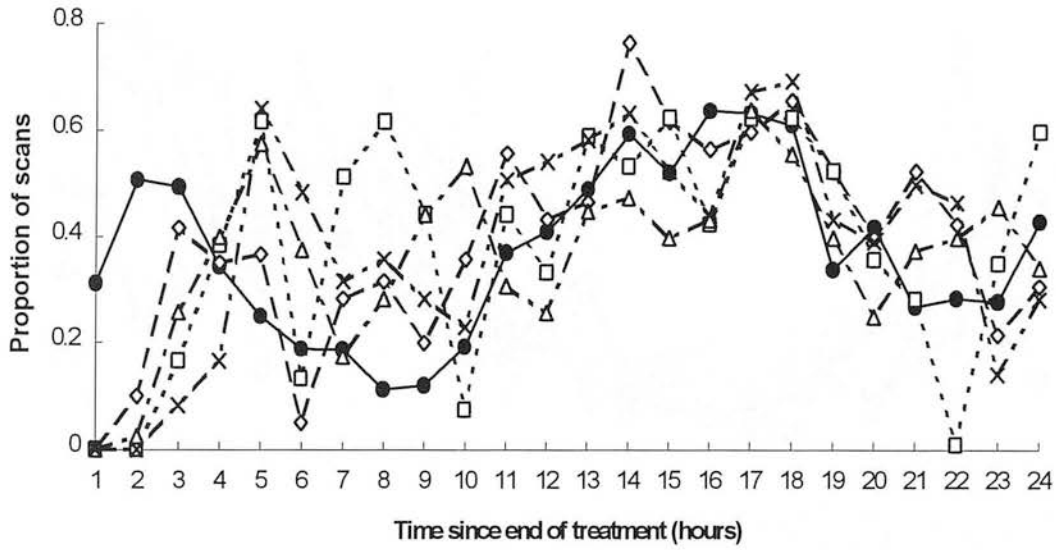

Figure 4.6. Effect of four lairage treatments on the proportion of scans spent ruminating post-treatment compared with the equivalent time pre-treatment (n=6). For legends, see Figure 4.4.

Resting behaviour

Figure 4.7 shows the mean proportion of scans spent standing post-treatment compared with the equivalent pre-treatment. For the first 2h post-treatment, there was no significant effect of lairage treatment. In the third hour after unloading, those that had been given a 12h lairage period spent a lower proportion of scans standing (0·41 s.e. 0·08) and a greater proportion of scans lying (0·59 s.e. 0·08) than those from the other three lairage groups (standing:- 3h lairage: 0·86 s.e. 0·05; 3h vehicle: 0·68 s.e. 0·10; no lairage: 0·92 s.e. 0·05; lying:- 3h lairage: 0·14 s.e. 0·05; 3h vehicle: 0·32 s.e. 0·10; no lairage: 0·08 s.e. 0·05).

There was an increase in the proportion of scans spent standing and a decrease in the proportion of scans spent lying compared with the equivalent time pre-treatment (Table VI, n=6 sheep per group, $p<0·0001$, but the results from the

individual sheep were not fully independent). These changes lasted for 3h for the sheep that had been confined on a moving vehicle and for 4h for the sheep that had been confined in a stationary vehicle. This difference from pre-treatment levels in both standing and lying lasted longer for those that had not been given a period of lairage (4h), than for those that had 3h access to food and water on/off the vehicle (3h), or those that had been given a 12h lairage period (2h).

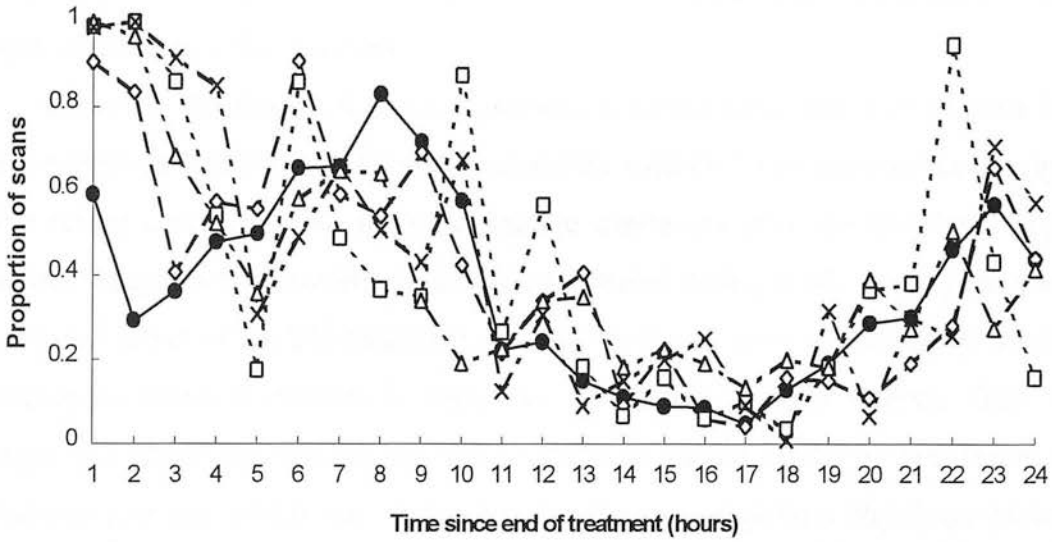

Figure 4.7. Effect of four lairage treatments on the proportion of scans spent standing post-treatment compared with the equivalent time pre-treatment (n=6). For legends, see Figure 4.4.

4.4 Discussion

Post-treatment, the sheep that had not had access to food and water during the treatment had a higher hay intake than sheep from the other three lairage treatments, and initially they spent a greater proportion of scans eating than the sheep given a 12h lairage period. This suggests that, whilst those that were only given 3h access to food (either on or off the vehicles) had a lower hay intake than those given 12h access to food, providing food and water reduced the effects of the 24h journey in terms of post-treatment food intake. In addition, the post-treatment increase in the proportion of scans spent eating compared with pre-treatment levels lasted longer for the groups not given a lairage period. These results may indicate a greater effect of a 24h journey on the groups that had no lairage period compared with those that were

fed and watered during the journey, in terms of changes in behaviour and food intake.

Initially, there was no difference between the four lairage treatments in the proportion of scans spent eating post-treatment. All sheep showed an increase compared with the equivalent time pre-treatment. The large proportion of scans spent eating (almost 100%), and the decrease in the proportion of scans spent ruminating may suggest that the initial motivation to feed is similar regardless of the length of time since the last meal.

However, the change from pre-treatment in eating behaviour was shortest for the sheep given 12h lairage. This, in conjunction with the lower proportion of scans spent eating compared with the other lairage treatments after the first hour post-treatment suggests that providing a 12h lairage period during a 24h journey lessened the overall effect of the 24h treatment, as it resulted in a quicker recovery from the treatment in terms of changes in behaviour and food and water intakes. The 3h lairage period off the vehicle resulted in the next fastest return to pre-treatment behaviour patterns, which was faster than for the groups given a 3h lairage period on the vehicle. This would suggest that providing a period of lairage off the vehicle will reduce the overall effect of a long journey on the behaviour of sheep.

4.4.1 Effect of feeding on/off vehicles

The lower proportion of scans eating and lying and the greater proportion of scans standing during the lairage period by the sheep that were kept on the vehicle compared with those that were unloaded would suggest that the space restriction on the vehicles was inhibiting behaviour, reducing lying and restricting the ability of the sheep to reach the food compared with those unloaded to a higher space allowance. However, there was little difference in the mean hay intakes for sheep fed and watered on the vehicles compared with the groups that were unloaded for 3h (mean hay intake/sheep: unloaded: 0·47kg; vehicle: 0·42kg). Sheep fed and watered on the vehicle spent a greater proportion of scans idling than those that were unloaded and also than at the equivalent time pre-treatment, which would support the idea that the performance of behaviours has been restricted.

The liveweight loss during transport was similar to that found by Warriss (1990) and Knowles *et al.* (1993). The smaller change in liveweight in those given a 12h lairage would suggest that a 12h lairage period was more effective in reducing the effects of a 24h journey than a 3h lairage period. The lack of difference in liveweight change during treatment between sheep given a 3h lairage period (either on or off the vehicle), and those not given a period of lairage may suggest that more than 3h is required to lessen any weight losses occurring as a result of food and water deprivation during transport.

Sheep given a 12h lairage period ate more hay and drank more water than those given a 3h lairage period off the vehicle, which may suggest that 3h was not sufficient for the sheep to eat and drink as much as they may have required. As sheep at pasture will spend 9-12h/day feeding in bouts lasting 20-90 minutes, followed by bouts of resting and ruminating lasting 45-90 minutes (Lynch *et al.*, 1992), the longer lairage will have allowed more bouts of feeding to occur. The hay intake over the first 3h of lairage is unlikely to have differed between these two groups, as no difference in the proportion of scans spent eating over the first 3h lairage was observed.

Previous work has shown that the majority of a sheep's water requirement is drunk within 3h of feeding (Ternouth, 1967; McKinley *et al.*, 1994), with an increase in the osmolality of both the rumen liquor and plasma occurring after feeding (Ternouth, 1967). For the sheep only given 3h access to feed and water, there may have been little opportunity to drink before the treatment continued, due to the high proportion of scans spent feeding. This would decrease the water available to be absorbed from the rumen to prevent any dehydration caused by the increased water requirement for saliva production, with an estimated 2·7 ml saliva swallowed with every 1g dry food (Stacy & Warner, 1966). Whilst the mean water intakes/sheep measured on the vehicles were the same as those measured off the vehicles (unloaded: 0·5 litres; vehicle: 0·5 litres), little drinking behaviour was observed in the back pens of the vehicles, and much of this recorded volume may have been due to spillage, as the water buckets on the vehicles were almost at floor level and sheep were frequently observed to stand in them.

Water intake in the four lairage groups was greater than for the control sheep over the first 12h post-treatment. While the control sheep had continuous access to food and water and may have been less susceptible to any dehydration caused by feeding, this increase may indicate that the sheep became dehydrated during the treatment, as although water intake has been observed to decrease during periods of food deprivation (Hecker *et al.*, 1964), a small volume of water is still drunk. The group fed and watered on the vehicle had a greater water intake post-treatment than the other two groups given food and water during the treatment. If the 3h period on the vehicle did not provide these sheep with the opportunity to drink, this will have increased the risk of them becoming dehydrated

The increase in the proportion of scans spent eating and standing and the corresponding decrease in the proportion of scans spent ruminating and lying seen over the 3h lairage periods were also seen during the 12h lairage period, but did not continue beyond the first 3h. This may suggest that a 3h lairage period is sufficient to satisfy any 'hunger' arising from a 12h food deprivation. For the group given a 12h lairage period, the difference in the proportion of scans in these behaviours from the equivalent time pre-treatment continued over the lairage, but it would appear from the figures that following the initial increase, the sheep go back to a cycle of behaviour similar to that seen pre-treatment, lagging by a few hours.

4.4.2 Effect of a second 12h treatment period

For sheep that were given a period of lairage, those that were confined on moving vehicles spent a lower proportion of scans ruminating during the second 12h of treatment than those confined on stationary vehicles, and than at the equivalent time pre-treatment. Combined with the greater proportion of scans spent standing and lower proportion of scans spent lying by all transported sheep compared with sheep confined in a stationary vehicle and with the equivalent time pre-treatment, it would seem that the motion of the vehicle is in some way affecting the behaviour of the sheep as has been found previously (Cockram *et al.*, 1996).

The increase in the proportion of scans moving seen during the first 2h of the second 12h treatment period could be indicative of an increase in investigation of the

surroundings by the sheep. With the proportion of scans spent ruminating showing fewer differences to pre-treatment (sheep confined on a stationary vehicle showed no difference to the proportion of scans spent ruminating at the equivalent time pre-treatment), this may suggest an increased adaptation to or familiarity with the environment (in this case the vehicles).

However, the group given a 12h lairage period showed differences in the proportion of scans spent standing and lying compared with the equivalent time pre-treatment for longer than the other 3 lairage treatments. This may suggest that being unloaded from the vehicle for a longer period of time has made them more unsettled and restless by the change in environment.

4.4.3 Post-treatment recovery

The increase in the proportion of scans spent feeding post-treatment agrees with previous work on the transport of sheep (Cockram *et al.*, 1996). The lower proportion of scans spent eating and the greater proportion of scans spent ruminating by sheep given a 12h lairage compared with the other lairage treatments and the less time taken to return to pre-treatment levels of eating and ruminating by these sheep, suggests that the provision of lairage did have an effect, reducing the impact of the total journey on the post-treatment behaviour of the sheep.

After the increase in the proportion of scans spent eating, there was an increase in the proportion of scans spent ruminating, which lasted longer for sheep that had been confined in a moving vehicle. As rumination is important for breaking food down to a size that can pass out of the rumen, the lower proportion of scans spent ruminating during treatment seen here and in other studies (Cockram *et al.*, 1996) may affect the rumen volume, potentially resulting in an increased tendency to ruminate. This may indicate a gut-fill effect on the performance of rumination. However, no effects of transport on post-treatment hay intake were found. During the lairage period, the sheep that had been confined on stationary vehicles were observed to spend a greater proportion of scans eating and a lower proportion of scans ruminating than the sheep that were confined on moving vehicles. The lack of difference may be in part due to the hay intake measurements being recorded over

12h for individually penned sheep, whilst the behaviour was recorded for group-housed sheep. Previous work has demonstrated an effect of social facilitation on the feeding of sheep (Tribe, 1950), which may have caused the group-housed sheep to spend more time feeding than the individually housed sheep.

The effects of transport and the associated food deprivation on the behaviour of sheep found in this experiment are consistent with those reported by others. In this experiment, confinement on a vehicle (both stationary and moving) increased the proportion of scans spent standing and reduced the proportion of scans spent lying and ruminating compared with the equivalent time pre-treatment. These changes have been reported by a number of authors (Ewbank & Kent, 1990; Cockram *et al.*, 1996). The post-treatment changes are also consistent with other work. Increases in feeding behaviour following transport and food deprivation have been reported (Knowles *et al.*, 1995; Cockram *et al.*, 1996; Hall *et al.*, 1997; Sibbald, 1997), with field trials reporting an increased interest in feeding following transport (Knowles *et al.*, 1994; 1996). This consistency of the results with previous work would suggest that despite the statistical non-independence of the individual experimental units used in the analysis, the results obtained are valid.

4.5 Conclusions

The provision of a period of lairage off the vehicle for 3h and 12h seems to facilitate post-transport recovery with decreased changes and shorter lengths of changes in behaviour and food and water intakes.

The destination of the lambs may be important in considering the overall benefits of providing a period of lairage during long journeys. For example, if the sheep are going straight to slaughter it may not be beneficial as liveweight loss has little effect on carcass weight and they may not be held in lairage long enough for the decreased effects on the observed changes in behaviours to outweigh exposing the sheep to additional handling by further unloading and reloading, which has been shown to be the cause of most pre-slaughter bruising (Cockram & Lee, 1991). If the sheep were going to market, a period of lairage could be beneficial as the sheep will

be exposed to additional stressors there and increasing the speed of recovery by breaking the journey to provide food and water may lessen the overall effects.

Providing food and water on the vehicles in this study was not satisfactory, with access to feed restricted by the lack of space. An increased feeding face may increase access for feeding, but the problem of allowing sufficient time for sheep to feed and drink must be considered.

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CHAPTER 5 Effect of familiarity of environment after a 16h journey on the behaviour of lambs.

Abstract

The commercial transportation of sheep involves moving the sheep to unfamiliar environments. An experiment was designed to investigate the effect of familiarity of post-transport housing following a 16h road journey on the behavioural responses of sheep. Behavioural observations were made on non-transported control lambs and transported lambs before, during and after 16h of transport (n=6). After the 16h treatment period, sheep were either moved or unloaded into a new pen, or left in or returned to their home pen.

During the treatment period, transported sheep spent a greater proportion of scans standing (0·75), and a lower proportion of scans lying (0·20) than control sheep (standing: 0·44; lying: 0·54). Sheep that had been kept outside in a paddock spent a greater proportion of scans standing (0·70), and a lower proportion of scans lying (0·28), than sheep that had been housed inside during transport (standing: 0·57; lying: 0·42).

Post-treatment, sheep that were moved to a different type of housing or an unfamiliar location spent a lower proportion of scans eating (different housing: 0·23; unfamiliar location: 0·32) than sheep that were returned to similar type of housing (0·46) or a familiar location (0·50). Sheep moved to a different type of housing or an unfamiliar location also took longer to return to pre-treatment levels of behaviour than sheep returned to a similar type of housing or a familiar location post-treatment.

Changes in diet and environment affected the behaviour of the sheep. The results from this study suggest that unloading sheep into novel environments may result in the sheep feeding less than they would in familiar surroundings, particularly

if the food is unfamiliar, and this may affect the time required to fully recover from a journey.

5.1 Introduction

The transportation of sheep involves a number of changes to a sheep's surroundings. These include loading onto vehicles, close confinement during a journey and unloading into an unfamiliar environment. Unfamiliar environments have been suggested as one of the most stressful aspects of pre-slaughter handling (Grandin, 1980). The effect that a novel environment may have on the recovery of sheep post-transport is not yet known.

A number of studies have investigated the adaptation of sheep to novel environments. McNatty *et al.* (1972) found that after sheep were transferred to a new environment, the concentration of plasma cortisol took 28 days to return to a basal level, suggesting adaptation to the new environment took place over a four week period.

Done-Currie *et al.* (1984) found that when lambs were transferred from pasture to housing, they did not consume their full daily food ration until 31 days after transfer. The change in diet may be partly responsible for this length of time, as sheep do not readily eat novel foods (Burritt & Provenza, 1989; Provenza *et al.*, 1995). Work by Fordham *et al.* (1991) examined behaviour and blood constituents (cortisol and beta-endorphins) of sheep moved to a novel environment. They suggested that the observed elevation in hormone concentrations and the changes in behaviour indicate that it may take even longer (up to 8/9 weeks) before sheep are fully adjusted to a new environment.

Both of these studies involved transferring sheep from pasture to housing, and found that sheep responded to the new environment by 'withdrawing' and spending more time in 'non-alert' behaviours (standing quietly, hanging head), and less time in 'alert' behaviours (observing humans and other sheep) than sheep that were familiar with the environment. It was suggested that this was an attempt by the 'new' sheep to alleviate the 'stress' of the new situation (Done-Currie *et al.*, 1984).

This experiment investigated the effect of exposure to a novel environment (both inside and outside) on the behaviour of sheep following a 16h road journey.

5.2 Material and methods

5.2.1 Animals and management

Forty-two 16-week-old lambs (Suffolk x Greyface) with liveweights between 30 and 40kg were used. One week prior to treatment, the lambs were randomly divided into 7 groups (n=6), balancing for liveweight and sex (3 females and 3 castrated males per group). All sheep were individually marked with a stockmarker (Super Sprayline Stockmarker, Ritchey Tagg Ltd.) to enable identification of each sheep during the behavioural observations. Four groups were housed in outside enclosures (area of each enclosure: 610·33m^2), on grass with *ad-libitum* water, and three groups were housed inside on straw (area of each pen: 6·25m^2), with *ad-libitum* hay and water. All pens were continuously lit by artificial lighting to allow overnight behavioural observations.

Table I. Allocation of groups to housing and treatment (n=6).

Group	Pre-treatment housing	Transport (h)	Post-treatment housing
1	outside paddock	0	same outside paddock
2	outside paddock	0	novel inside pen
3	inside pen	0	same inside pen
4	outside paddock	16	novel outside paddock
5	outside paddock	16	novel inside pen
6	inside pen	16	same inside pen
7	inside pen	16	novel inside pen

5.2.2 Treatments

Two transport treatments were investigated: 0h transport (control) during which sheep remained in their home pens, and 16h transport (transport) during which sheep were loaded onto a vehicle and driven on public roads for 16h.

Five housing treatments were investigated:

-outside grass paddock to novel grass paddock

-outside grass paddock to novel inside pen

-inside pen to same inside pen

-inside pen to novel inside pen

-outside paddock to same outside paddock

The allocation of groups to the transport and housing treatments is shown in Table I.

Groups 4, 5, 6 and 7 were transported on a single deck, non-articulated Ford Cargo six-cylinder diesel vehicle. The livestock area was divided into two pens: front pen (1·08m x 2·22m) that held groups 4 & 5, and a back pen (1·62m x 2·22m) that held groups 6 & 7, additional non-experimental sheep were loaded into the pens to give a space allowance of 0·2m^2/sheep in each pen.

Chequerplate flooring covered with wood-shavings was used in the transporter to simulate conditions of commercial sheep transportation. Observations were conducted from a separate observation area to cause minimal disturbance to the sheep and the pens were illuminated to allow observation at night.

5.2.3 Protocol

Behaviour of the sheep was recorded for 24h pre-treatment (06·00h-06·00h). At 15·00h on the treatment day, groups 4, 5, 6 and 7 were loaded onto the vehicle and driven on public roads for 16h, returning to the site every 3 hours to change the observers and drivers. The behaviour of the sheep was recorded on the vehicle and in the home pens for the control groups during the 16h treatment period. The treatment period was intended to be 15h, but due to the transport vehicle breaking down during treatment, the actual length of the treatment was 16h

On completion of the 16h journey, the sheep were unloaded into the housing as shown in Table I, and group 2 was loaded onto the vehicle and moved to a novel inside pen. The behaviour of the sheep was recorded by direct observation for 24 hours following unloading (07·00h-07·00h).

5.2.4 Behavioural observations

Behaviour of the sheep was recorded by direct observation before during and after treatment for all groups. Observations were recorded by scan sampling each sheep every 6 minutes for 24h pre-treatment, throughout the treatment (both in the shed and on the vehicle) and for the first 24h post-treatment. Observations for each sheep were recorded on hand-held computers (PSION LZ64) and analysed using behavioural software (Noldus Information Technology, 1993 and 1994).

The behaviours recorded by scans sampling were:

standing (upright stationary posture)

walking (upright posture involving a change in location)

lying (recumbent posture with the body in contact with the floor)

dog-sitting (rear quarters in contact with floor, like a dog)

eating (ingestion of food followed by jaw movements and swallowing)

ruminating (Regurgitation, chewing and reswallowing a bolus of food not immediately preceded by eating)

foraging (nosing through bedding)

investigating environment (sniffing/licking/chewing pen or solid structure)

investigating sheep (sniffing/licking/chewing another sheep)

idling (not performing any obvious behaviour)

other (performing another behaviour not described above).

Continuous observations were also carried out between 06·00h and 18·00h, when daylight permitted recording of sheep in the outside pens. The occurrence of the following behaviours were recorded for each sheep:

get up (change in posture from lying/dog-sitting to standing)

start drinking (immersing muzzle in water for >5 seconds)

butting (hitting another sheep with head)

5.2.5 Hay and water intake

The hay and water intakes of the group penned sheep were recorded for two consecutive 12h periods post-treatment immediately following the end of the 16h

treatment period. Known amounts of hay and water were offered every 12h and refusals were measured at the end of each 12h period.

5.2.6 Statistical analysis

The statistical analysis of data generally requires the independence of the experimental units involved. This would require either the individual penning of sheep or an increase in the number of groups used in this experiment. While individual penning would provide data more suitable for statistical analysis, work has shown that this can adversely affect the behaviour of sheep (Marsden & Wood-Gush, 1986) with individually penned sheep consuming less food than those housed in groups (Webster *et al.*, 1972). This may reduce the benefit of applying any findings to the commercial situation, as sheep are housed and transported in groups and their behaviour will therefore be influenced by conspecifics. This reduction in feeding and the potential adverse effects on behaviour make it preferable to use group-housed sheep when recording behaviour to ensure that (as far as possible in an experimental situation) the sheep behave as they would under commercial conditions. To do this would require the use of an increased number of sheep to increase the number of replicates in each treatment. This was not financially feasible during this project. However, an increasing number of experiments on sheep transport have been published, which allows us to compare the consistency of the results in terms of the effect of transport and the associated food deprivation.

As a result of the non-independence of the data from the behavioural observations, the majority of the results from the analysis will be reported descriptively, some significant effects will be reported. In both cases, any consistency with published work will be commented on.

For each sheep, the proportion of scans spent in each behaviour was calculated for every hour of the observation periods. These proportions were used for statistical analysis (n=6). A repeated measures analysis of variance (Laird & Ware, 1982), using the mixed procedure within SAS version 6 (SAS Institute Inc. Cary USA) was used to examine the effects of group and time on the proportion of scans spent in each behaviour. Where there were interactions between treatment or post-treatment

housing and time, the differences between the least-square means were examined, comparing: the 16h treatment period and the equivalent time pre-treatment; the first 12h post-treatment and the equivalent time pre-treatment; the second 12h post-treatment and the equivalent time pre-treatment, and the first and second 12h post-treatment periods. This analysis was repeated to examine the effects of treatment (0h transport and 16h transport), pre-treatment housing (inside/outside), post-treatment housing (inside/outside, familiar/unfamiliar location, similar/different to pre-treatment type of housing) and time on the proportion of scans spent in each behaviour.

Due to the design of this experiment not being orthogonal, it was not possible to examine interactions between treatment and housing. It may be important to investigate the effect of such interactions in another study, as they may affect the relevance of the results found in this experiment. For example, during the pre-treatment period differences were found in the proportion of scans spent eating, lying, idling, ruminating and standing by sheep housed inside and outside pre-treatment (Table II). This effect of pre-treatment housing may also have influenced the mean response of sheep to transport and any change in housing, particularly as the design of the experiment was unbalanced (with an unequal number of groups being allocated to each treatment), which may result in any differences confounding the effects of the treatments under investigation. A more appropriate design would have been to have a balanced design investigating fewer treatments (e.g. one type of pre-treatment housing) and perform further experiments to investigate the others.

The total incidence of getting up for each sheep was calculated for 6h periods for the pre-treatment and post-treatment observations and for the duration of the 16h treatment period. These were analysed using non-parametric tests (n=6). The effects of time (Wilcoxon signed ranks) treatment and housing (inside/outside, familiar/unfamiliar location, similar/different type of housing to pre-treatment) (Mann-Whitney) on the incidence of getting up were examined (Siegel & Castellan, 1988). This was repeated for the incidences of drinking and butting. As for the analysis of variance, no interactions between treatment and housing were examined due to the design of the experiment not being orthogonal.

Table II. Mean proportion of scans (s.e.) spent performing each behaviour during the 24h pre-treatment period for sheep housed inside and outside (n=6).

	Inside	Outside
dogsitting	0	0·001 (0·001)
eating	0·32 (0·01)	0·48 (0·01)
foraging	0·01 (0·003)	0·01 (0·004)
idling	0·21 (0·01)	0·27 (0·01)
investigating environment	0·01 (0·001)	0·02 (0·003)
investigating sheep	0·005 (0·001)	0·01 (0·003)
lying	0·57 (0·01)	0·44 (0·01)
other	0·01 (0·001)	0·01 (0·003)
ruminating	0·44 (0·01)	0·25 (0·01)
standing	0·42 (0·01)	0·53 (0·01)
walking	0·005 (0·001)	0·03 (0·003)

Table III. Mean proportion of scans (s.e.) spent performing each behaviour during 16h treatment period and the equivalent time pre-treatment for control and transported sheep (n=6).

		Pre-treatment	During treatment
dogsitting	control	0	0
	transported	0·001 (0·001)	0·01 (0·003)
foraging	control	0·01 (0·005)	0·05 (0·01)
	transported	0·02 (0·004)	0·03 (0·01)
idling	control	0·31 (0·02)	0·37 (0·03)
	transported	0·25 (0·01)	0·90 (0·01)
investigating environment	control	0·02 (0·005)	0·06 (0·01)
	transported	0·01 (0·004)	0·04 (0·01)
investigating sheep	control	0·01 (0·005)	0·05 (0·01)
	transported	0·004 (0·001)	0·04 (0·01)
lying	control	0·54 (0·02)	0·54 (0·02)
	transported	0·53 (0·01)	0·20 (0·03)
other	control	0·01 (0·005)	0·05 (0·01)
	transported	0·006 (0·001)	0·05 (0·01)
ruminating	control	0·32 (0·02)	0·29 (0·03)
	transported	0·34 (0·02)	0·12 (0·01)
standing	control	0·44 (0·02)	0·44 (0·02)
	transported	0·45 (0·02)	0·76 (0·04)
walking	control	0·02 (0·01)	0·02 (0·005)
	transported	0·02 (0·004)	0

5.3 Results

5.3.1 During treatment

Table III shows the mean proportion of scans spent in each behaviour during the 16h treatment period and the equivalent time pre-treatment.

Feeding behaviour

As reported by other authors (Ewbank & Kent, 1990; Cockram *et al.*, 1996), during the treatment period, control sheep spent a greater proportion of scans ruminating than transported sheep (Table III, Figure 5.1). During the treatment period, transported sheep spent a lower proportion of scans ruminating than during the equivalent time pre-treatment (Table III).

During the final 5h of the treatment period, sheep that had been kept inside spent a greater proportion of scans ruminating (0·53 s.e. 0·03) than sheep that had been kept outside (0·20 s.e. 0·02).

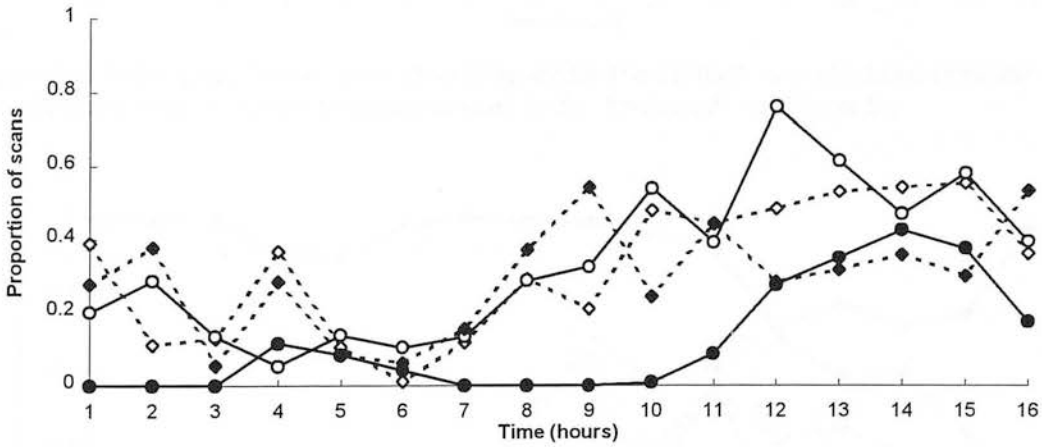

Figure 5.1. Mean proportion of scans spent ruminating during 16h treatment period (closed symbols) and the equivalent time pre-treatment (open symbols) (n=6). ◆ - control ● - transported.

Resting behaviour

As expected, during the treatment period, sheep that were being transported spent a greater proportion of scans standing and a lower proportion of scans lying than control sheep and than during the equivalent time pre-treatment (Table III, Figure 5.2). Sheep that had been housed outside spent a greater proportion of scans

standing (0·70 s.e. 0·05) and a lower proportion of scans lying (0·28 s.e. 0·04) than those that had been kept inside (standing: 0·57 s.e. 0·05; lying: 0·42 s.e. 0·05).

Other behaviour

During the 16h treatment period, transported sheep spent a greater proportion of scans idling than control sheep and than at the equivalent time pre-treatment (Table III, Figure 5.3).

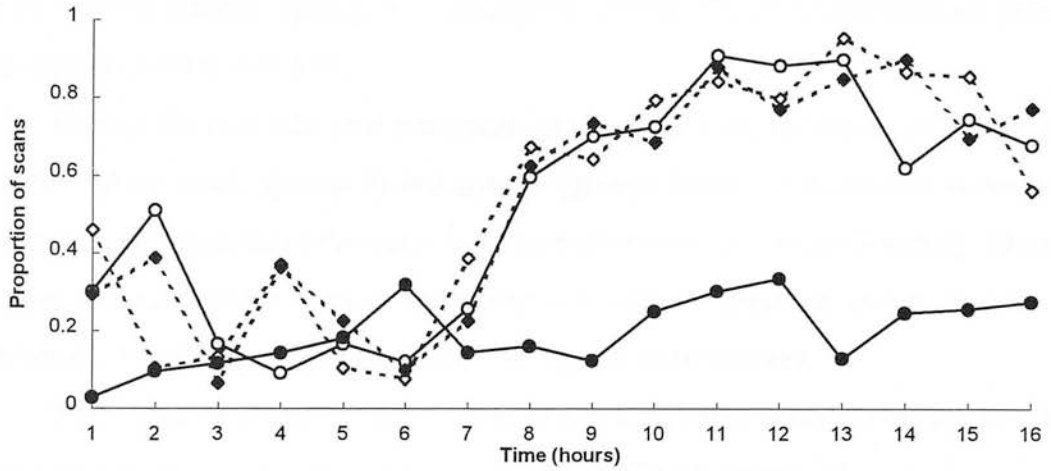

Figure 5.2. Mean proportion of scans spent lying during 16h treatment period (closed symbols) and the equivalent time pre-treatment (open symbols) (n=6). For legends, see Figure 5.1.

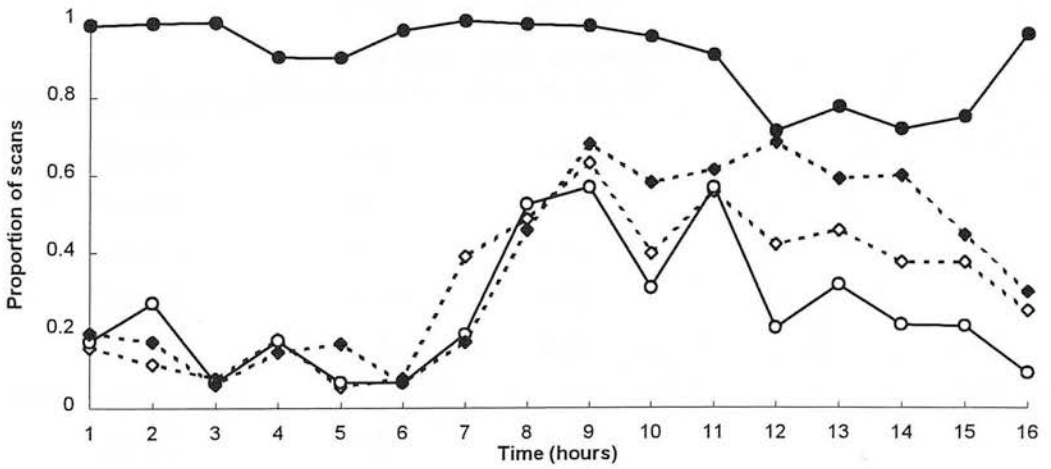

Figure 5.3. Mean proportion of scans spent idling during 16h treatment period (closed symbols) and the equivalent time pre-treatment (open symbols) (n=6). For legends, see Figure 5.1.

5.3.2 Post-treatment

Hay and water intakes

Table IV shows the mean hay and water intakes/sheep for the groups housed inside post-treatment. No analysis of these results was possible as the means were obtained from group intakes. The groups that had been kept outside pre-treatment (groups 2 and 5) ate the least hay during the first 12h post-treatment. The groups that had been housed inside pre-treatment (groups 3, 6 and 7) ate similar amounts of hay during the first 12h post-treatment, with no effect of either transport (groups 6 & 7) or moving location (group 7). During the second 12h post-treatment all groups ate similar quantities of hay.

During the first 12h post-treatment, groups that were moved to an unfamiliar location (from inside (group 7) and outside (groups 2 and 5)) drank less water than those that were housed in the same location post-treatment (groups 3 and 6). During the second 12h post-treatment, the sheep that were transported and moved to an unfamiliar location (groups 5 & 7) had the highest water intakes.

There was no change in the number of drinking bouts observed during the first 12h post-treatment compared with the equivalent 12h pre-treatment.

Table IV. Mean hay and water intakes/sheep during two consecutive 12h periods post-treatment for sheep housed inside (n=6).

	1st 12h post-treatment	2nd 12h post-treatment
Mean Hay Intake (kg)		
Group 2	0·36	0·47
Group 3	0·74	0·42
Group 5	0·40	0·43
Group 6	0·88	0·48
Group 7	0·78	0·51
Mean Water Intake (l)		
Group 2	1·67	0·83
Group 3	3·33	0·67
Group 5	2·50	1·50
Group 6	3·00	0·50
Group 7	2·17	1·50

Tables V and VI show the mean proportions of scans spent performing each behaviour during the two 12h periods post-treatment and the equivalent 12h periods pre-treatment for sheep in similar/different types of housing (Table V) and in familiar/unfamiliar locations (Table VI) post-treatment.

Table V. Mean proportion of scans (s.e.) spent performing each behaviour post-treatment and during the equivalent time pre-treatment for sheep moved to a similar or different type of housing post-treatment (n=6).

		Pre treatment		Post treatment	
		1st 12h	2nd 12h	1st 12h	2nd 12h
dogsitting	similar	0	0·001 (0·001)	0	0
	different	0	0	0	0
eating	similar	0.47 (0·02)	0·30 (0·02)	0·46 (0·02)	0·27 (0·01)
	different	0·60 (0·03)	0·34 (0·02)	0·23 (0·02)	0·26 (0·03)
foraging	similar	0·01 (0·004)	0·02 (0·006)	0·01 (0·002)	0·02 (0·007)
	different	0·004 (0·002)	0·004 (0·002)	0·02 (0·006)	0·04 (0·02)
idling	similar	0·01 (0·002)	0·01 (0·004)	0·18 (0·02)	0·36 (0·01)
	different	0·004 (0·002)	0·003 (0·002)	0·38 (0·04)	0·38 (0·03)
investigating environment	similar	0·01 (0·002)	0·02 (0·005)	0·01 (0·002)	0·03 (0·006)
	different	0·01 (0·004)	0·01 (0·003)	0·03 (0·006)	0·05 (0·02)
investigating sheep	similar	0·006 (0·002)	0·008 (0·004)	0·003 (0·001)	0·02 (0·006)
	different	0·004 (0·002)	0·003 (0·002)	0·01 (0·005)	0·04 (0·02)
lying	similar	0·46 (0·01)	0·60 (0·02)	0·43 (0·02)	0·69 (0·01)
	different	0·29 (0·01)	0·55 (0·01)	0·62 (0·03)	0·69 (0·01)
other	similar	0·008 (0·002)	0·01 (0·004)	0·005 (0·001)	0·03 (0·007)
	different	0·009 (0·003)	0·01 (0·003)	0·01 (0·005)	0·05 (0·02)
ruminating	similar	0·36 (0·02)	0·38 (0·02)	0·33 (0·02)	0·38 (0·02)
	different	0·16 (0·02)	0·31 (0·01)	0·37 (0·02)	0·41 (0·02)
standing	similar	0·52 (0·01)	0·39 (0·02)	0·54 (0·01)	0·30 (0·01)
	different	0·65 (0·01)	0·43 (0·02)	0·37 (0·03)	0·31 (0·02)
walking	similar	0·02 (0·004)	0·008 (0·003)	0·03 (0·006)	0·008 (0·002)
	different	0·07 (0·007)	0·02 (0·003)	0·007 (0·002)	0·008 (0·002)

Feeding behaviour

There was no effect of transport on the proportion of scans spent eating during the first 4h post-treatment. From 5-12h post-transport, transported sheep spent a lower proportion of scans eating (0·34 s.e. 0·02) than control sheep (0·43 s.e. 0·04).

During the first 12h post-treatment, sheep that were in a similar type of housing post-treatment (but not necessarily in the same location), spent a greater proportion of scans eating than sheep that were moved to a different type of housing (Table V, Figure 5.4) (n=6 sheep per group, $p<0{\cdot}0001$, but results from individual sheep were not fully independent). During the 24h post-treatment period, sheep that were in a different type of housing post-treatment spent a lower proportion of scans eating than at the equivalent time pre-treatment (Table V). Sheep that were housed in similar accommodation spent a greater proportion of scans eating during the first 2h post-treatment (0·69 s.e. 0·02) compared with the equivalent time pre-treatment (0·43 s.e. 0·02). There was no effect of moving the sheep to an unfamiliar location post-treatment on the proportion of scans spent eating or ruminating post-treatment.

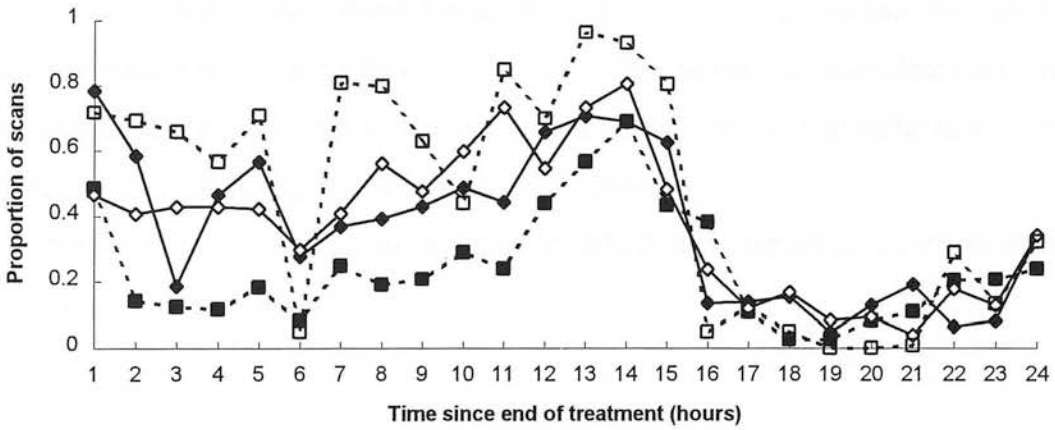

Figure 5.4. Effect of the similarity of housing post-treatment on the mean proportion of scans spent eating post-treatment for sheep housed in similar and different types of accommodation post-treatment (closed symbols) and the equivalent period pre-treatment (open symbols) (n=6). ◆ similar type of housing post-treatment, ■ different type of housing post-treatment.

During the first 12h post-treatment sheep that were in familiar housing (same location as pre-treatment) spent a greater proportion of scans eating than sheep that were moved to an unfamiliar location post-treatment (Figure 5.5, Table VI) (n=6 sheep per group, $p<0{\cdot}0001$, but results from individual sheep were not fully independent). Post-treatment, sheep that were moved to an unfamiliar pen/enclosure spent a lower proportion of scans eating than at the equivalent time pre-treatment (Table VI). During the first 2h post-treatment, sheep that were returned to a familiar

location spent a greater proportion of scans eating (0·64 s.e. 0·02) than during the equivalent time pre-treatment (0·38 s.e. 0·03).

There was no difference in the proportion of scans spent ruminating during the first hour post-treatment between sheep housed inside (0·05 s.e. 0·02) and outside (0·04 s.e. 0·02). During the next 20h, sheep that were housed inside post-treatment spent a greater proportion of scans ruminating (0·43 s.e. 0·01) than those that were housed outside post-treatment (0·25 s.e. 0·02).

No differences were found in the proportion of scans spent ruminating during the first hour post-treatment between sheep in either similar or different types of housing post-treatment. During the remaining observations post-treatment, sheep housed in different type of housing post-treatment spent a greater proportion of scans ruminating than at the equivalent time pre-treatment (Figure 5.6, Table V). During the first 2h post-treatment, sheep housed in similar housing post-treatment spent a lower proportion of scans ruminating (0·15 s.e. 0·02) than at the equivalent time pre-treatment (0·41 s.e. 0·04), but from 4h post-treatment there were no differences from the proportion of scans spent ruminating pre-treatment.

None of the treatments were found to effect the proportion of scans spent foraging post-treatment.

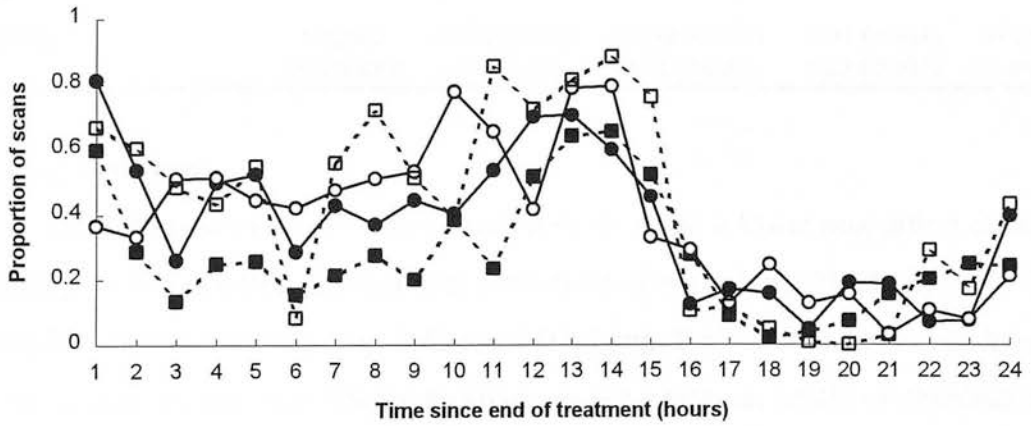

Figure 5.5. Effect of familiarity of post-treatment housing on the mean proportion of scans spent eating post-treatment (closed symbols) compared with the equivalent time pre-treatment (open symbols) (n=6). ● familiar housing, ■ unfamiliar housing.

Table VI. Mean proportion of scans (s.e.) spent performing each behaviour post-treatment and during the equivalent time pre-treatment for sheep moved to a familiar or unfamiliar location post-treatment (n=6).

		Pre treatment		Post treatment	
		1st 12h	2nd 12h	1st 12h	2nd 12h
dogsitting	familiar	0	0	0	0
	unfamiliar	0	0·001	0	0
eating	familiar	0·48 (0·02)	0·29 (0·02)	0·50 (0·03)	0·27 (0·02)
	unfamiliar	0·52 (0·03)	0·33 (0·02)	0·32 (0·03)	0·27 (0·02)
foraging	familiar	0·02 (0·006)	0·01 (0·007)	0·005 (0·002)	0·01 (0·008)
	unfamiliar	0·004 (0·002)	0·01 (0·005)	0·02 (0·003)	0·02 (0·01)
idling	familiar	0·002 (0·001)	0·01 (0·006)	0·17 (0·01)	0·34 (0·01)
	unfamiliar	0·008 (0·002)	0·002 (0·001)	0·30 (0·03)	0·36 (0·02)
investigating environment	familiar	0·008 (0·002)	0·01 (0·007)	0·01 (0·002)	0·02 (0·007)
	unfamiliar	0·02 (0·003)	0·01 (0·005)	0·02 (0·004)	0·03 (0·01)
investigating sheep	familiar	0·002 (0·001)	0·01 (0·006)	0·002 (0·001)	0·01 (0·008)
	unfamiliar	0·008 (0·002)	0·002 (0·001)	0·008 (0·003)	0·02 (0·01)
lying	familiar	0·45 (0·02)	0·63 (0·02)	0·44 (0·03)	0·69 (0·02)
	unfamiliar	0·39 (0·02)	0·55 (0·02)	0·52 (0·03)	0·69 (0·01)
other	familiar	0·007 (0·002)	0·02 (0·006)	0·004 (0·001)	0·02 (0·007)
	unfamiliar	0·009 (0·002)	0·007 (0·002)	0·01 (0·003)	0·03 (0·01)
ruminating	familiar	0·35 (0·02)	0·37 (0·03)	0·32 (0·03)	0·37 (0·02)
	unfamiliar	0·27 (0·03)	0·35 (0·02)	0·35 (0·02)	0·37 (0·02)
standing	familiar	0·54 (0·02)	0·37 (0·02)	0·54 (0·02)	0·30 (0·02)
	unfamiliar	0·57 (0·02)	0·43 (0·02)	0·46 (0·02)	0·30 (0·01)
walking	familiar	0·009 (0·002)	0·009 (0·004)	0·01 (0·004)	0·005 (0·002)
	unfamiliar	0·05 (0·006)	0·01 (0·002)	0·02 (0·007)	0·01 (0·002)

Resting behaviour

From 4h post-treatment, sheep housed inside spent a lower proportion of scans standing (0·35 s.e. 0·01), and a greater proportion of scans lying (0·62 s.e. 0·01) than sheep housed outside (standing: 0·48 s.e. 0·02; lying: 0·43 s.e. 0·02). Sheep housed inside post-treatment took longer to return to pre-treatment levels of standing and lying (17h) than sheep housed outside (12h).

Sheep that were housed in a familiar location post-treatment spent a lower proportion of scans lying and a greater proportion of scans standing during the first

12h post-treatment than sheep that had been moved to an unfamiliar location (Table VI).

During the first 12h post-treatment, sheep in similar type of housing post-treatment spent a lower proportion of scans lying (Figure 5.7, Table V) and a greater proportion of scans standing than sheep that had been moved to a different type of housing (n=6 sheep per group, $p<0.0001$, but the results from the individual sheep were not fully independent).

During the first 2h post-treatment, sheep in similar type of housing post-treatment spent a lower proportion of scans lying (0·24 s.e. 0·02), and a greater proportion of scans standing (0·74 s.e. 0·01) compared with the equivalent time pre-treatment (lying: 0·47 s.e. 0·01; standing: 0·51 s.e. 0·03, Figure 5.7). These groups had returned to pre-treatment levels of lying and standing by 6h post-treatment. Sheep that were moved to a different type of housing post-treatment spent a greater proportion of scans lying and a lower proportion of scans standing than at the equivalent time pre-treatment during the first 12h post-treatment, and had returned to pre-treatment levels by 17h post-treatment.

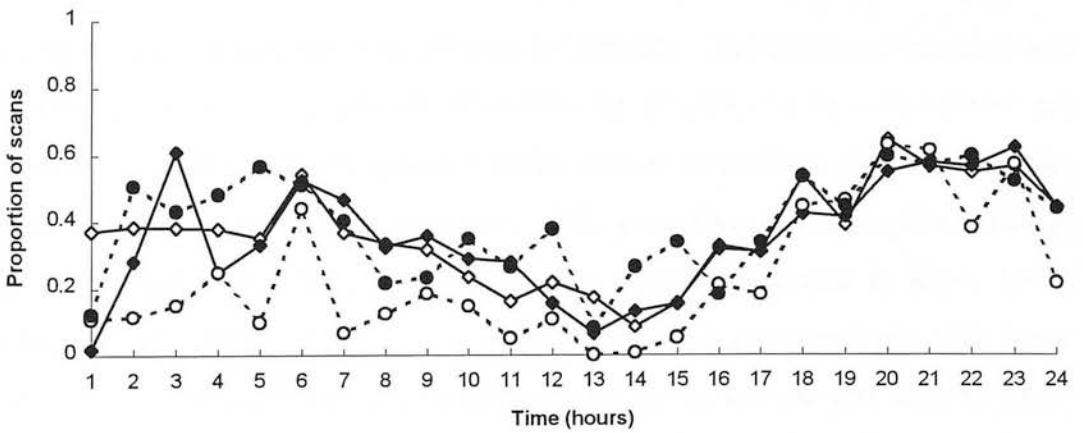

Figure 5.6. Effect of similarity of post-treatment housing on the proportion of scans spent ruminating post-treatment (closed symbols) and the equivalent time pre-treatment (open symbols) (n=6). ◆- similar type of housing ●- different type of housing.

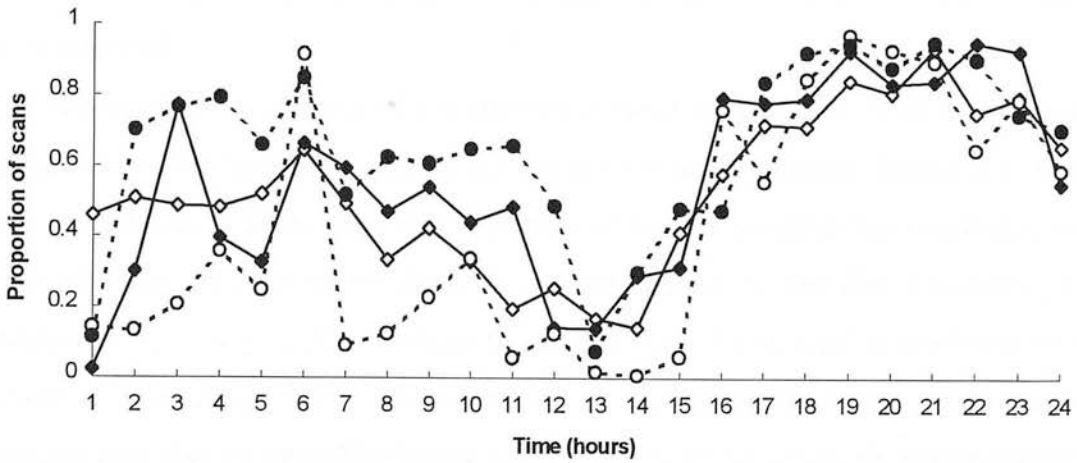

Figure 5.7. Effect of type of housing post-treatment on proportion of scans spent lying post-treatment (closed symbols) compared with the equivalent time pre-treatment (open symbols) (n=6). ◆- similar type of housing ●- different type of housing.

5.4 Discussion

As said previously, care should be taken when interpreting the results of this study, as the group-housing of the sheep used for behavioural observations may have affected the independence of the behaviour of the individual sheep. An improvement of the experimental design would be to increase the number of groups used for each treatment. This would have greatly increased the cost of the study and the time required to perform it, which was not feasible. The effects of transport and the associated food deprivation on the behaviour of sheep in this experiment are consistent with those found by others. Confinement on a moving vehicle reduced the amount of lying and ruminating and increased the proportion of scans spent standing. This has been reported by a number of other authors (Ewbank & Kent, 1990; Cockram *et al.*, 1996). The post-treatment changes are also consistent with other work. The increase in feeding seen when given access to food has been reported following transport and food deprivations (Knowles *et al.*, 1995; Cockram *et al.*, 1996; Hall *et al.*, 1997; Sibbald, 1997) and field trials have reported sheep have an increased interest in feeding following transport (Knowles *et al.*, 1994; 1996). The consistency of the results from this experiment with previous work would suggest that despite the statistical non-independence of the individual experimental units

used in the analyses, the results give a strong indication as to the effects of the treatments used.

Pre-treatment experience of hay affected post-treatment intake, with the groups that had been on grass pre-treatment having the lowest hay intakes during the first 12h post-treatment. There have been a number of studies investigating neophobia in sheep, showing them to be unwilling to eat novel foods in familiar surroundings (Chapple *et al.*, 1987), and less willing to eat such foods in unfamiliar environments (Burritt & Provenza, 1997). The lower hay intake of sheep moved inside post-treatment may also be caused by inexperience with the novel foodstuff, sheep moved onto pasture with no experience of grazing were found to still be eating more slowly than experienced grazers after 15 weeks at pasture (Arnold & Maller, 1977).

Sheep that were moved from an inside pen to a different inside pen post-treatment consumed similar amounts of hay to those housed in the same inside pen which agrees with the work of Burritt & Provenza (1997), who found that lambs moved to unfamiliar locations did not decrease their consumption of a familiar food.

As well as the lower food intakes in groups moved inside, sheep that were moved to unfamiliar locations or different types of housing post-treatment spent a lower proportion of scans eating during the first 12h post-treatment than those returned to familiar locations or a similar type of housing. This has implications for the welfare of sheep during transport, as being moved to a novel environment is a major aspect of the transportation process.

Current transport regulations require that animals are watered and if necessary fed after 14h of transport, with a only a limited time (1h) being required for this mid-journey lairage period (Welfare of Animals (Transport) Order, 1997). Whilst previous work has shown that sheep will readily feed during periods of lairage (Cockram *et al.*, 1997), the sheep in that study were returned to familiar home pens and fed a familiar food. The results from this study suggest that unloading sheep to novel environments results in the sheep feeding less than they would in more familiar surroundings particularly if the food is also unfamiliar. This may reduce any benefit such a break in the journey may have on the overall effect of the food and water deprivation experienced during the transportation process. It also

increases the time taken for sheep to recover post-transport at their final destination. In this study, sheep that were moved to different types of housing took 16h to return to pre-treatment levels of eating, whilst sheep returned to a similar type of housing recovered in less time (2h). It would seem that unloading sheep into similar types of housing at the end of a journey with familiar forms of feed facilitates the recovery from the journey.

A change in location was also found to decrease the water consumption of the sheep in this study. Other work has reported that lambs will not readily drink from an unfamiliar source even when they have been deprived of water (Nichols, 1944; Knowles *et al.*, 1993) No difference in the number of observed drinking bouts was found between familiar/unfamiliar locations, so the intake of water during each bout must have been less for sheep moved to an unfamiliar location. As all sheep were housed on the same experimental site it is unlikely that there was any difference in the water other than a change in container. Such neophobia will be particularly important if sheep are fed a dry food, as they will have a greater requirement for 'free' water than those on fresh herbage, although their total water intake (including the water content of the feed) has been shown to be higher than for those on fresh herbage (Calder *et al.*, 1964). Studies have shown sheep to drink most of their requirement immediately after feeding (Ternouth, 1967; McKinley *et al.*, 1994), with an increase in the osmolality of both the rumen liquor and plasma occurring after feeding (Ternouth, 1967). Knowles *et al.* (1993) reported increased plasma osmolality during the first 12h in lairage when sheep were observed to feed but had low water intakes. Neophobia of a new water supply or novel container may cause increased dehydration as the sheep begin to feed.

5.4.1 Behaviour during transport

Pre-treatment housing had an effect on the behaviour of sheep during transport, with sheep that had been housed inside spending a greater proportion of scans ruminating than those that had been housed outside. Sheep that had been penned inside also spent a greater proportion of scans foraging, walking and lying and a lower proportion of scans standing during transport than sheep that had been housed

outside. The fact that these sheep had been housed inside, more closely confined than those housed outside may partly explain this, as the sheep may have become more accustomed to the close proximity of other sheep.

5.4.2 Post-treatment recovery

When sheep and cattle are provided with fresh food, there is an increase in feeding behaviour, even when animals are fed *ad-libitum* (Winter & Hillerton, 1995; Sibbald, 1997). This may partly explain the lack of difference in feeding behaviour between control and transported treatments during the first 4h post-treatment, as all groups (except the control group kept in the same outside paddock) were presented with 'fresh' food, in the form of fresh hay for measuring hay intake for housed sheep, and those outside were unloaded/moved into a paddock. Previous work has demonstrated an effect of social facilitation on feeding in sheep with the presence of a hungry animal increasing the food intake of a non-deprived animal (Tribe, 1950; Rook & Penning, 1991). This may also explain the similar amount of feeding behaviour seen for both control and transported sheep during the first 4h post-treatment, the sight of the transported sheep feeding may have initiated feeding in the control groups or caused them to continue eating.

Differences in eating behaviour account for most of the changes in resting behaviour, increased eating behaviour corresponds to increases in standing, with increased lying and ruminating occurring when decreases in the proportion of scans spent eating were observed. Sheep housed inside post-treatment seemed to change from eating to resting sooner than those housed outside post-treatment. The food source may be partly responsible for this, and the restricted access to the hay racks may have increased the rate of intake due to competition. Jenkins & Leymaster (1987) found that increased competition for access to a feeding stall resulted in sheep making fewer visits to the feeding stall, but compensated for this by increasing the amount consumed during and the length of each visit. Work has shown that there is no difference in intake or eating time when grass and hay are fed as forage (Dulphy, 1972; Peterson *et al.*, 1974). However, the fact that sheep housed outside in this trial are grazing rather than feeding from a container enables them to select their diet over

the whole paddock, which is likely to increase the time spent feeding compared with sheep housed inside where hay is only available in one place.

5.5 Conclusions

Changes in environment post-treatment affected the post-treatment behaviour of sheep, with sheep moved to unfamiliar locations or different types of housing taking longer to return to pre-treatment levels of behaviour (17h) than sheep returned to a familiar location or a similar type of housing (2-6h). The differences found in this study may not be truly representative of the effects of commercial transportation as all sheep were returned to the same experimental site, with food and water from the same sources as pre-treatment. The increased lying behaviour seen in those moved to different and unfamiliar environments followed the decreased eating observed by these groups. This has implications for the recovery of sheep following transport. Knowles *et al.* (1993) reported that 144h were required for sheep to recover fully (for changes in biochemical measures of fasting and dehydration to return to normal levels) from a road journey of 24h to France. The results from this study may suggest that the long period of recovery reported by Knowles *et al.* (1993) may be due in part to an unwillingness to feed in a novel environment, as sheep in that study were moved from hill grazing in Scotland to lairage pens in the south of England.

These results have implications for the welfare of sheep during transport as changes in environment will occur as they are moved to markets and slaughterhouses. To increase speed of recovery from transport (in terms of behavioural changes), moving sheep to a similar type of housing (housed to housed, field to field) with a similar type of food may improve post-transport welfare.

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CHAPTER 6 Effect of an additional period of food deprivation following a 15h journey on the behaviour of sheep.

Abstract

On arrival at slaughterhouses within the UK, sheep can be exposed to additional periods of food deprivation after those already experienced during transport. Little is currently known about how this additional period of food deprivation affects the animal's behaviour. An experiment was designed to investigate the effect of 12h of food deprivation following a 15h road journey on the behavioural responses of sheep (n=6). Behavioural observations were made on lambs (35kg) before, during and after either 15h transport or 15h remaining in the home pens (controls). Following the 15h treatment period, sheep were either fed or deprived of food for 12h.

During the 15h treatment period, transported sheep spent a lower proportion of scans ruminating (0·03) and lying (0·22), and a greater proportion of scans standing (0·78) and idling (0·96) than sheep that were not transported (ruminating: 0·36; lying: 0·64; standing: 0·35; idling: 0·36).

Sheep without access to food for the first 12h post-treatment had a lower number of drinking bouts (2), and had lower water intakes over the first 12h post-treatment (1·5l) than sheep with access to food (drinking bouts: 5; water intake: 1·9l). All groups showed an increase in the proportion of scans spent eating after being fed post-treatment. During the first 12h post-treatment, sheep without access to food spent a greater proportion of scans foraging (0·06) and investigating the environment (0·07) than sheep with access to food (foraging: 0·003; investigating environment: 0·02). Sheep without access to food also spent a greater proportion of

scans lying (0·75), and got up from a lying position more often (17·5) than sheep with access to food during the first 12h post-treatment (lying: 0·51; get up: 14). During the first 12h post-treatment more butting was observed (5) than during the equivalent time pre-treatment (0).

Withholding food after 15h transport was associated with an increase in food-seeking behaviour which decreased after food was provided. This may indicate a potential welfare problem in that it suggests that the sheep were motivated to find food. However, an additional period of food deprivation following transport did not appear to affect recovery from transport (in terms of time taken to return to pre-treatment levels of behaviour).

6.1 Introduction

Large numbers of sheep are transported by road in the UK each year, to markets, slaughter-houses and for export to mainland Europe. Over recent years, there has been increased public concern regarding the welfare of animals in transit, with public demonstrations resulting in some ferry companies banning the carrying of livestock on their ferries. This, and the ban on the live export of calves from the UK has disrupted live sheep exports, with numbers decreasing each year (Meat and Livestock Commission, 1996a, b; 1997a, b).

Since the Farm Animal Welfare Council report on priorities in animal welfare research (FAWC, 1988) identified transport as a priority, more work has been performed to investigate the behavioural and physiological responses of sheep to transport. These studies have examined a range of factors, including space allowance during transport (Cockram *et al.*, 1996), and journey times (Knowles *et al.*, 1993; 1994).

During transport, sheep have been observed to spend less time lying and ruminating than when not transported (Knowles *et al.*, 1993; Cockram *et al.*, 1996). Increased heart rate and plasma cortisol concentrations have been reported during 12h road journeys (Cockram *et al.*, 1996). After longer journeys to mainland Europe (18h and 24h), changes indicative of dehydration (increased plasma osmolality, albumin and total protein), and the metabolism of body reserves (high levels of

plasma beta-hydroxybutyrate, free fatty acids and urea) were found (Knowles *et al.*, 1994).

Previous UK regulations require sheep to be fed every 15h during a journey, unless the final destination can be reached within 16h (Welfare of Animals During Transport Order, 1994). On arrival at slaughter-houses in the UK, regulations require the provision of food on arrival and then every 12h, unless the sheep are going to be slaughtered within 12h of arrival. This allowed a potential food withdrawal of up to 28h (Welfare of Animals (Slaughter or Killing) Regulations, 1995). New legislation that came into force in July 1997 (Welfare of Animals (Transport) Order, 1997) allows journeys of up to 14h that may be followed by an additional 14h journey after a 1h stoppage for resting, watering and if necessary feeding animals.

Sheep have been observed to be primarily interested in feeding after unloading (Knowles *et al.*, 1994), spending an increased amount of time feeding over the first few hours post-treatment than under normal conditions (Cockram *et al.*, 1996). With a long period (144h) required for the full recovery of blood metabolites from the changes seen during 9h-14h journeys (Knowles *et al.*, 1993), the failure to provide feed immediately on arrival may have additional effects on the recovery of sheep from transport. Work by Warriss *et al.* (1989) found that a number of blood biochemical variables (packed cell volume, liver glycogen, plasma free fatty acid and beta-hydroxybutyrate concentrations) were significantly altered by 24h fasting of sheep, with plasma concentrations of beta-hydroxybutyrate increasing progressively over 72h food withdrawal.

This experiment was performed to investigate the effects of a 15h road journey and an additional 12h period of food deprivation after the journey on the behaviour of sheep. In particular it examined whether the additional period of food deprivation altered the time required for the sheep to return to pre-treatment levels of behaviour.

6.2 Material and methods

6.2.1 Animals and management

The experiment used 48 16-week old lambs (Suffolk x Greyface), with liveweights of between 30kg and 40kg. The lambs had been weaned and kept at pasture on site before the experiment. All lambs were naive of the transportation process. One week prior to the experiment, the sheep were housed to allow some acclimatisation to take place. The sheep were randomly allocated to 8 groups, each containing 6 animals, balancing for sex and liveweight. All sheep were individually marked with a stockmarker (Super Sprayline Stockmarker, Ritchey Tagg Ltd.) to enable identification of each sheep during the behavioural observations.

Behaviour sheep (groups 1-4) were housed in groups (2·5m x 2·5m) on wood shavings, with *ad-libitum* access to known amounts of hay and water. Sheep used for blood-sampling (groups 5-8) were individually penned (2·0m x 1·5m) on wood shavings, with *ad-libitum* access to known amounts of hay and water. The sheep were provided with known amounts of hay (individual pens: 1kg; group pens: 5kg) and water (individual pens: 10 litres; group pens: 60 litres) twice a day, at 06·00h and at 18·00h.

The building was lit throughout the study by artificial lighting, and ventilated by Yorkshire boarding on one wall of the building and a ridge inlet in the roof.

6.2.2 Treatments

Four treatments were used, with one group of behaviour and one group of blood-sampling sheep being allocated to each treatment (Table I). Two transport treatments were used, 0h transport during which the sheep remained in their home pens with access to known amounts of food and water (C), and 15h transport, during which the sheep were loaded onto a vehicle and transported for 15h (T).

Two post-treatment feeding regimes were also used, *ad-libitum* where the sheep had *ad-libitum* access to known amounts of hay and water at the end of the treatment (F1), and 12h food withdrawal, where for the first 12h after the end of the treatment period, sheep had no access to food but *ad-libitum* access to a known

amount of water (F2). At the end of this 12h period, all sheep were given a known amount of hay.

Table I. Allocation of sheep to transport and post-treatment food withdrawal treatments.

Treatment	Groups	Transport	Post-treatment food withdrawal
CF1	1, 5	0h	0h
CF2	2, 6	0h	12h
TF1	3, 7	15h	0h
TF2	4, 8	15h	12h

Groups 2 and 4 were visually isolated from groups 1 and 3, and groups 6 and 8 were visually isolated from groups 5 and 7 when housed in the shed. The groups were visually isolated as previous work has shown that the sight of sheep feeding was stressful to sheep without access to food (Wronska *et al.*, 1990).

Groups 3, 4, 7 and 8 were transported on a single deck, non-articulated Ford Cargo six-cylinder diesel vehicle. The livestock area was divided into two pens: front pen (1·08m x 2·22m) that held the blood sampled sheep (groups 7 & 8), and a back pen (1·62m x 2·22m) that held the behaviour sheep (groups 3 & 4) and six additional non-experimental sheep, giving a space allowance of 0·2m^2/sheep in each pen.

Chequerplate flooring covered with wood-shavings was used in the transporter to simulate conditions of commercial sheep transportation. Observations were conducted from a separate observation area to cause minimal disturbance to the sheep and the pens were illuminated to allow observation at night.

6.2.3 Protocol

At the start of the treatment period, groups 7 and 8 were loaded into the front pen of the vehicle (blood sampled groups), and groups 3 and 4 were loaded into the rear pen of the vehicle (behaviour groups). The sheep were transported for 15h

(15.00h - 06.00h), returning to the experimental site every 3h for blood sampling and to change the observers and driver.

On completion of the 15h journey, the sheep were returned to their home pens. Groups 1, 3, 5, and 7 were provided with a known amount of hay (as for pre-treatment), groups 4 and 8 were not provided with hay, and groups 2 and 6 had any remaining hay removed from their pens. All groups were provided with known amounts of water every 12h (as for pre-treatment). Twelve hours post-treatment, all groups were provided with known amounts of hay (as for pre-treatment).

6.2.4 Liveweight

The liveweights of the behaviour sheep were measured using a weigh-crate before the start of the 15h treatment period, at the end of the 15h treatment period, 12h post-treatment and 24h post-treatment.

6.2.5 Hay and water intake

The hay and water intakes of both the individually and group penned sheep were recorded for two consecutive 12h periods pre-treatment (at the same time as the pre-treatment behavioural observations), and for two consecutive 12h periods post-treatment. Known amounts of hay and water were offered every 12h and refusals were measured at the end of each 12h period.

6.2.6 Behavioural observations

Behaviour of the group pens was recorded by direct observation from an elevated platform in the shed before and after treatment for all groups, and during treatment for groups 1 and 2 (control). Observations were recorded by scan sampling each sheep every 6 minutes for 24h pre-treatment, throughout the treatment (both in the shed and on the vehicle) and for the first 24h post-treatment. Observations for each sheep were recorded on hand-held computers (PSION LZ64) and analysed using behavioural software (Noldus Information Technology, 1993 and 1994).

The behaviours recorded by scan sampling were:

standing (upright stationary posture)

walking (upright posture involving a change in location)

lying (recumbent posture with the body in contact with the floor)

dogsitting (rear quarters in contact with floor, like a dog)

eating (ingestion of food followed by jaw movements and swallowing)

ruminating (regurgitation, jaw movements and reswallowing that is not preceded by eating)

foraging (nosing through bedding)

investigating environment (sniffing/licking/chewing pen or solid structure)

investigating sheep (sniffing/licking/chewing another sheep)

idling (performing no apparent behaviour)

other (performing another behaviour not described above).

Continuous observations were also carried out over these periods. The occurrence of the following behaviours were recorded continuously for each sheep during observations in the shed:

get up (change in posture from lying/dog-sitting to standing)

start drinking (immersing muzzle in water for >5 seconds)

butting (butting another sheep with head)

6.2.7 Statistical analysis

The statistical analysis of data generally requires the independence of the experimental units involved. This would require either the individual penning of sheep or an increase in the number of groups used in this experiment. Some sheep in this experiment were individually penned, these sheep were blood sampled as part of the larger project that this reported experiment was part of. These sheep were also used to obtain information on the food and water intakes of the sheep during the experiment. While the data obtained was more suitable for statistical analysis, work has shown that sheep housed individually (and also when isolated) consume less food than those housed in groups (Webster *et al.*, 1972). This means that any results

obtained are likely to underestimate the intakes of sheep of a similar size in the commercial situation. Individual penning has also been shown to adversely affect the behaviour of sheep (Marsden & Wood-Gush, 1986), which would reduce the benefit of applying any findings to the commercial situation, as sheep are housed and transported in groups and their behaviour will therefore be influenced by conspecifics. This reduction in feeding and the potential adverse effects on behaviour make it preferable to use group-housed sheep when recording behaviour to ensure that (as far as possible in an experimental situation) the sheep behave as they would under commercial conditions. To do this would require the use of an increased number of sheep to increase the number of replicates in each treatment. This was not financially feasible during this project. However, an increasing number of experiments on sheep transport have been published, which allows us to compare the consistency of the results in terms of the effect of transport and the associated food deprivation.

As a result of the non-independence of the data from the behavioural observations, the majority of the results from the analysis will be reported descriptively, some significant effects will be reported. In both cases, any consistency with published work will be commented on.

For each sheep, the proportion of scans spent in each behaviour was calculated for every hour of the observation periods. These proportions were used for statistical analysis (n=6). A repeated measures analysis of variance (Laird & Ware, 1982), using the mixed procedure within SAS version 6 (SAS Institute Inc. Cary USA) was used to examine the effects of treatment (control and transport), post-treatment feeding regime (F1 and F2), and time on the proportion of scans spent in each behaviour. Where there were interactions between treatment, post-treatment feeding regime and time, the differences between the least-square means were examined, comparing: the 15h treatment period and the equivalent time pre-treatment; the first 12h post-treatment and the equivalent time pre-treatment; the second 12h post-treatment and the equivalent time pre-treatment, and the first and second 12h post-treatment periods.

Post-treatment hay and water intakes were analysed using a two-way ANOVA, examining the effects of treatment and post-treatment feeding. Liveweights were analysed using a two-way analysis of covariance, using pre-treatment liveweight as the covariate. In both cases, where there were significant effects, the significant differences between pairs of treatments were found by calculating the least significant differences (Snedecor & Cochran, 1980). The change in liveweight from pre-treatment was analysed using a paired t-test.

The total incidence of getting up for each sheep was calculated for 6h periods for the pre-treatment and post-treatment observations and for the duration of the 15h treatment period. These were analysed using non-parametric tests (n=6). The effects of time (Wilcoxon signed ranks) treatment and post-treatment feeding (Kruskal-Wallis) on the incidence of getting up were examined. Where significant effects were found, multiple comparisons were made between each pair of treatments, comparing the difference in ranks to the critical difference (Siegel & Castellan, 1988). This was repeated for the incidences of drinking and butting.

6.3 Results

6.3.1 During treatment

Table II shows the mean proportion of scans spent performing each behaviour during treatment and the equivalent 15h period pre-treatment for control and transported sheep.

Feeding behaviour

During the final 8h of the treatment period, transported and control sheep spent similar proportion of scans eating (transported 0 control 0·03 s.e. 0·02). There were few differences between treatments in the proportion of scans spent foraging during treatment.

Transported sheep spent a lower proportion of scans ruminating during treatment than control sheep (Figure 6.1, Table II), and than at the equivalent time pre-treatment (Table II). During the first hour of the treatment period control sheep

spent a lower proportion of scans ruminating (0·12 s.e. 0·04) than at the equivalent time pre-treatment (0·35 s.e. 0·06).

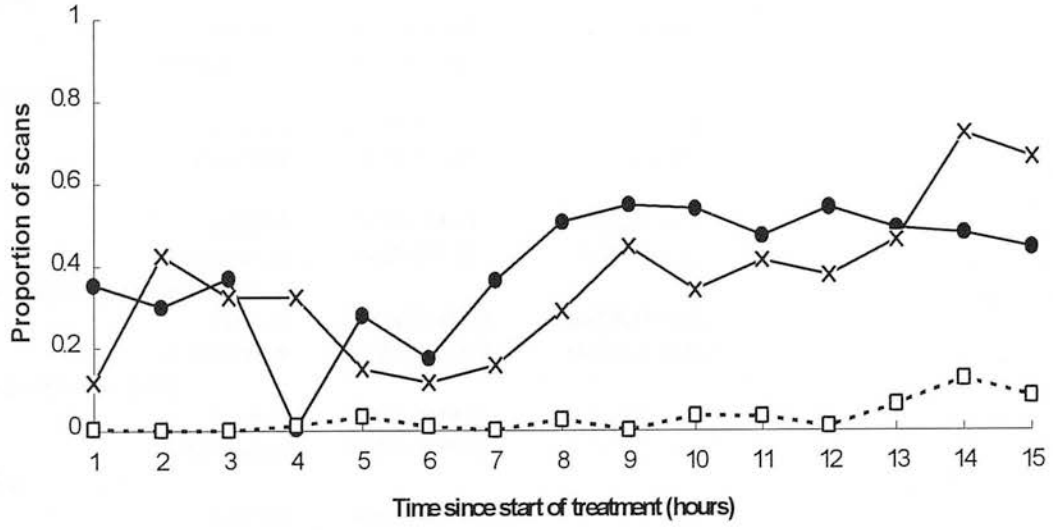

Figure 6.1. Effect of 15h transport on the mean proportion of scans spent ruminating during treatment (n=6). ●- pre-treatment (averaged over all groups); □- T, X- C.

Resting behaviour

Figure 6.2 shows the proportion of scans spent standing by sheep in each treatment. As expected, during the treatment period transported sheep spent a lower proportion of scans lying and a greater proportion of scans standing than control sheep (Table II). Transported sheep spent a lower proportion of scans lying and a greater proportion of scans standing than at the equivalent time pre-treatment during most of the treatment period (Table II).

There were no treatment effects on the proportion of scans spent walking. During the final few hours of the treatment period transported sheep spent a greater proportion of scans dogsitting than control sheep (Table II), but on each occasion, only one sheep was observed to be dogsitting at a time.

Table II. The mean proportion of scans (s.e.) performing each behaviour during 15h treatment and the equivalent time pre-treatment (n=6).

		Pre-treatment	During treatment
dogsitting			
	control	0	0
	transported	0	0·003 (0·001)
eating			
	control	0·26 (0·01)	0·27 (0·01)
	transported	0·28 (0·01)	-
foraging			
	control	0·003 (0·001)	0·02 (0·001)
	transported	0·001 (0·001)	0·001 (0·001)
idling			
	control	0·33 (0·02)	0·36 (0·02)
	transported	0·32 (0·03)	0·96 (0·01)
investigating environment.			
	control	0·004 (0·002)	0·008 (0·002)
	transported	0·008 (0·003)	0·008 (0·002)
investigating sheep			
	control	0·002 (0·001)	0·001 (0·001)
	transported	0·002 (0·001)	0·007 (0·003)
lying			
	control	0·66 (0·02)	0·64 (0·01)
	transported	0·64 (0·02)	0·22 (0·06)
other			
	control	0·003 (0·002)	0·006 (0·002)
	transported	0·002 (0·001)	0·001 (0·001)
ruminating			
	control	0·40 (0·02)	0·36 (0·02)
	transported	0·39 (0·03)	0·03 (0·01)
standing			
	control	0·33 (0·01)	0·35 (0·01)
	transported	0·39 (0·03)	0·78 (0·06)
walking			
	control	0·007 (0·002)	0·008 (0·003)
	transported	0·006 0·002)	0·002 (0·001)

Other behaviours

During the 15h treatment period, transported sheep spent a greater proportion of scans idling than control sheep and than at the equivalent time pre-treatment (Figure 6.3, Table II).

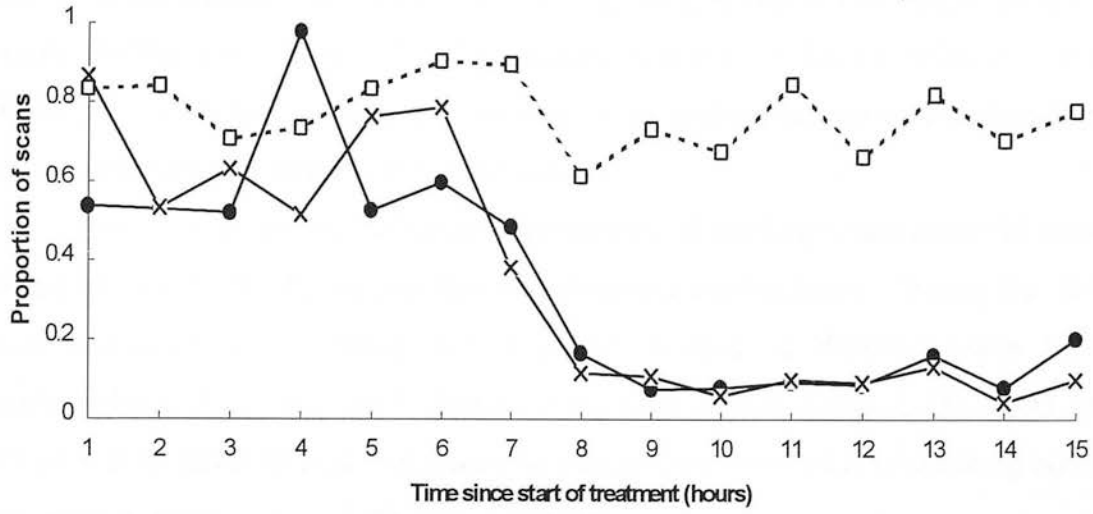

Figure 6.2. Effect of 15h transport on the mean proportion of scans spent standing during treatment and the equivalent time pre-treatment (n=6). For legends see Figure 6.1.

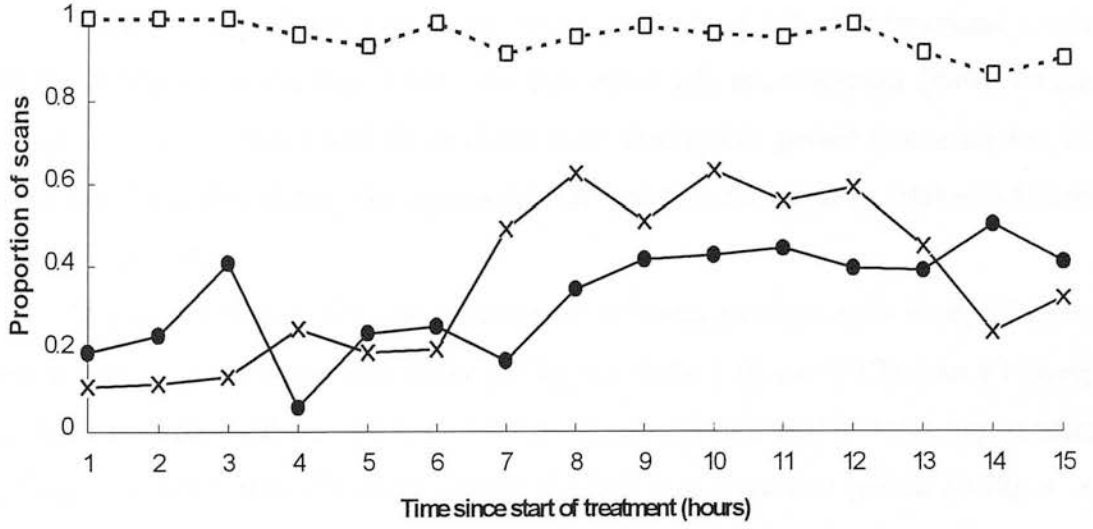

Figure 6.3. Effect of 15h transport on the mean proportion of scans spent idling during treatment and the equivalent time pre-treatment (n=6). For legends see Figure 6.1.

6.3.2 Post-treatment

On unloading, there was a significant effect of transport on the mean liveweight (adjusted means: transported 33·1kg s.e.. 0·71; non-transported 34·5kg s.e. 0·65, $p<0·05$). At 12h post-treatment, there was a significant effect of the post-

treatment feeding regime, with F1 sheep having a higher mean liveweight (adjusted mean: 36·0kg, s.e. 072) than F2 sheep (adjusted mean: 33·8kg s.e. 0·76, $p<0·05$). There was no effect of transport or post-treatment feeding regime on the change in liveweight during or after the treatment period.

There was no difference between the number of drinking bouts observed post-transport and the number during the equivalent time pre-treatment. During the first 12h post-treatment, F1 sheep had a greater number of drinking bouts (12h median/sheep: 5, Q1 3·5, Q3 8) than F2 sheep (12h median/sheep: 2, Q1 0, Q3 0). There was no effect of food withdrawal or transport on the number of drinking bouts observed during the second 12h post-treatment.

During the first 12h post-treatment, F1 sheep had a lower mean hay intake (0·3 kg s.e. 0·06) than over the equivalent 12h pre-treatment (0·5 kg s.e. 0·03, $p<0·01$). There was no significant difference in the mean water intake during the first 12h period post-treatment compared with the equivalent 12h period pre-treatment.

Sheep ate significantly more hay during the second 12h post-treatment (mean intake: 0·5kg s.e. 0·04) than during the equivalent 12h pre-treatment (mean intake: 0·4kg s.e. 0·03, $p<0·01$), and sheep drank more during this period (mean intake: 1·0 litres s.e. 0·14) than during the equivalent 12h pre-treatment (mean intake: 0·4 litres s.e. 0·14, $p<0·001$).

During the second 12h post-treatment after being provided with food, F2 sheep had higher intakes of hay and water (0·7kg s.e. 0·04; 1·4l s.e. 0·17) than F1 sheep (0·3kg s.e. 0·03; 0·5l s.e. 0·11, $p<0·001$). F1 sheep had higher total hay intakes (0·8kg s. e. 0·07) than F2 sheep during the 24h post-treatment period (0·7kg s. e. 0·03, $p<0·05$).

Feeding behaviour

Table III shows the mean proportion of scans spent performing each behaviour during the first 24h post-treatment and the equivalent time pre-treatment. As expected, during the first 4h post-treatment, F1 sheep spent a greater proportion of scans eating (0·53 s.e. 0·03) than at the equivalent time pre-treatment (0·23 s.e. 0·02, Figure 6.4) (n=6 sheep per group, $p<0·0001$, but results from individual sheep were

not fully independent). During the first 12h post-treatment F2 sheep spent a lower proportion of scans eating than F1 sheep and than at the equivalent time pre-treatment (Table III). When provided with hay, from 14-16h post-treatment F2 sheep spent a greater proportion of scans eating (0·89 s.e. 0·02) than at the equivalent time pre-treatment (0·36 s.e. 0·05), and a greater proportion of scans eating than F1 sheep (0·42 s.e. 0·04).

Table III. The mean proportion of scans (s.e.) performing each behaviour post-treatment and the equivalent time pre-treatment for sheep fed *ad-libitum* following 15h treatment (F1) or food-deprived for 12h (F2) (n=6).

		Pre treatment		Post treatment	
		1st 12h	2nd 12h	1st 12h	2nd 12h
dogsitting					
	F1	0	0	0	0
	F2	0	0	0	0
eating					
	F1	0·31 (0·02)	0·23 (0·01)	0·43 (0·02)	0·24 (0·01)
	F2	0·30 (0·02)	0·26 (0·01)	0	0·37 (0·01)
foraging					
	F1	0·001 (0·001)	0	0·003 (0·002)	0·007 (0·007)
	F2	0·004 (0·002)	0	0·06 (0·01)	0
idling					
	F1	0·15 (0·01)	0·35 (0·02)	0·15 (0·03)	0·31 (0·03)
	F2	0·27 (0·03)	0·33 (0·03)	0·60 (0·03)	0·26 (0·03)
investigating environment.					
	F1	0·01 (0·002)	0·006 (0·002)	0·02 (0·003)	0·003 (0·002)
	F2	0·01 (0·004)	0·005 (0·003)	0·07 (0·01)	0·004 (0·002)
investigating sheep					
	F1	0·002 (0·001)	0·001 (0·001)	0·001 (0·001)	0·001 (0·001)
	F2	0·002 (0·001)	0	0·01 (0·005)	0·001 (0·001)
lying					
	F1	0·60 (0·02)	0·72 (0·01)	0·51 (0·02)	0·72 (0·02)
	F2	0·58 (0·02)	0·68 (0·02)	0·75 (0·02)	0·57 (0·02)
other					
	F1	0·002 (0·002)	0·002 (0·001)	0·006 (0·002)	0·002 (0·002)
	F2	0·004 (0·002)	0·002 (0·001)	0·004 (0·002)	0·005 (0·002)
ruminating					
	F1	0·53 (0·01)	0·41 (0·02)	0·40 (0·02)	0·45 (0·03)
	F2	0·42 (0·01)	0·40 (0·03)	0·26 (0·02)	0·36 (0·02)
standing					
	F1	0·39 (0·02)	0·28 (0·01)	0·48 (0·02)	0·28 (0·02)
	F2	0·41 (0·02)	0·32 (0·02)	0·23 (0·01)	0·43 (0·02)
walking					
	F1	0·006 (0·002)	0·006 (0·002)	0·007 (0·003)	0·004 (0·002)
	F2	0·009 (0·002)	0·004 (0·002)	0·02 (0·005)	0·001 (0·001)

Between 8 and 12h post-treatment, F2 sheep spent a greater proportion of scans foraging (0·13 s.e. 0·03) than F1 sheep (0·003 s.e. 0·002), and than at the equivalent time pre-treatment (0·01 s.e. 0·003, Figure 6.5) (n-6 sheep per group, $p<0·0001$, but results from individual sheep were not fully independent).

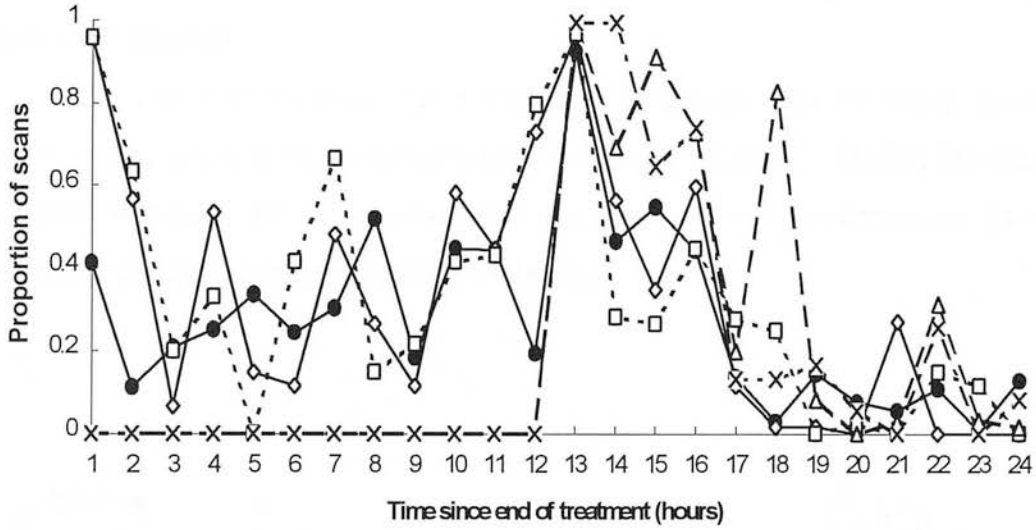

Figure 6.4. Effect of 15h transport and 12h food withdrawal post-treatment on the mean proportion of scans spent eating post-treatment compared with the equivalent time pre-treatment (n=6). ●- pre-treatment (averaged over all groups); ◊- CF1: 0h transport, 0h food withdrawal; □- TF1: 15h transport, 0h food withdrawal; Δ- CF2: 0h transport, 12h food withdrawal; X- TF2: 15h transport, 12h food withdrawal.

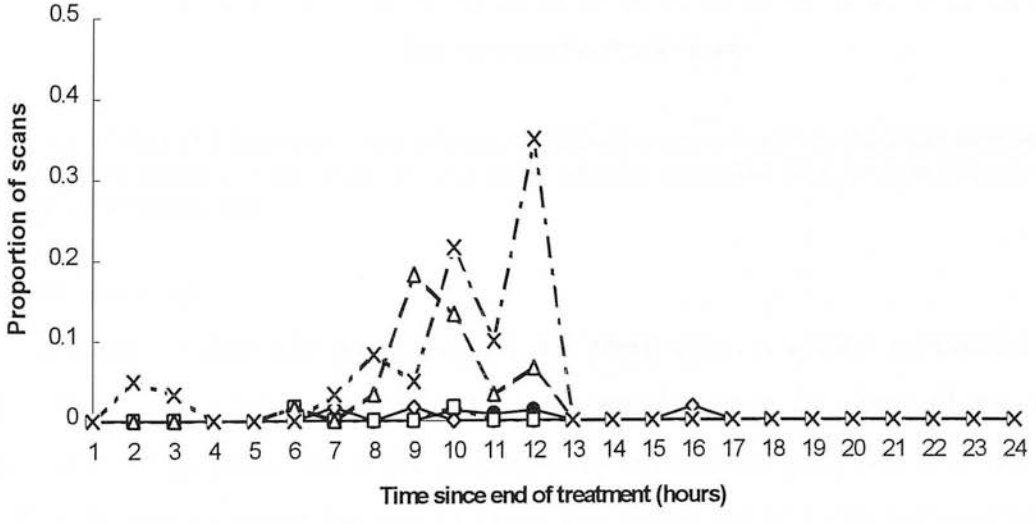

Figure 6.5. Effect of 15h transport and 12h food withdrawal post-treatment on the mean proportion of scans spent foraging post-treatment compared with the equivalent time pre-treatment (n=6). For legends see Figure 6.4.

During the first 2h post-treatment, all groups spent a lower proportion of scans ruminating than at the equivalent time pre-treatment (Figure 6.6). This decrease in the proportion of scans spent ruminating continued for 10h for both control sheep and F2 sheep. During the first 12h post-treatment, the proportion of scans spent ruminating by F1 sheep was greater than the proportion of scans spent ruminating by F2 sheep (Table III).

From 13h post-treatment (after F2 sheep had been fed) F1 sheep spent a greater proportion of scans ruminating than F2 sheep (Table III). During the second 12h post-treatment, F1 sheep showed little change from pre-treatment in the proportion of scans spent ruminating (Table III).

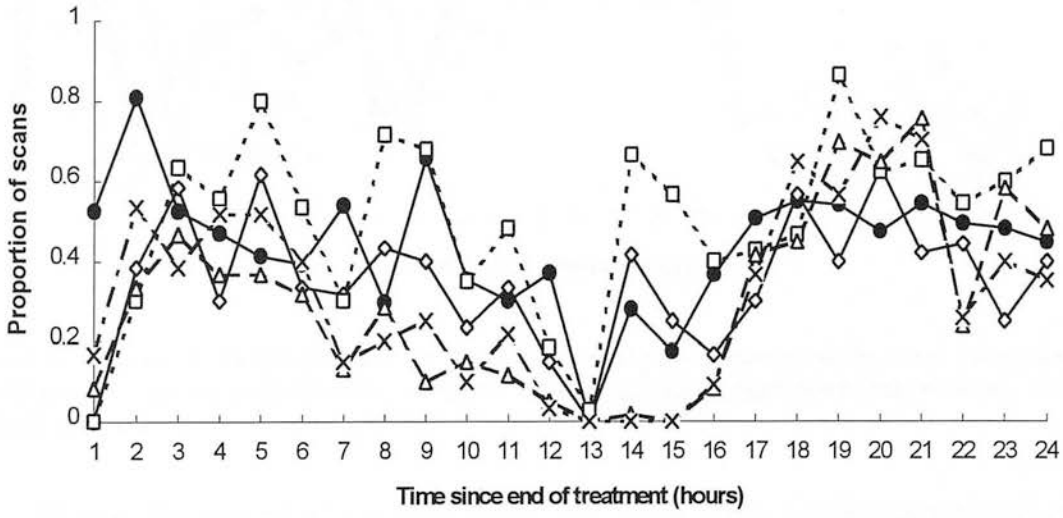

Figure 6.6. Effect of 15h transport and 12h food withdrawal post-treatment on the mean proportion of scans spent ruminating post-treatment compared with the equivalent time pre-treatment (n=6). For legends see Figure 6.4.

Resting behaviour

During the first 12h post-treatment, F2 sheep spent a greater proportion of scans lying, and a lower proportion of scans standing than F1 sheep (Table III, Figure 6.7). This difference in the proportion of scans spent lying was seen during the first 7h post-treatment for control sheep, but during the first 12h post-treatment for transported sheep.

During the first 2h post-treatment, F1 sheep spent a lower proportion of scans lying (0·19 s.e. 0·03), and a greater proportion of scans standing (0·80 s.e. 0·03) than at the equivalent time pre-treatment (lying: 0·74 s.e. 0·04; standing: 0·26 s.e. 0·04). From 3h post-treatment, F2 sheep spent a greater proportion of scans lying (0·77 s.e. 0·01), and a lower proportion of scans standing (0·21 s.e. 0·01) than at the equivalent time pre-treatment (lying: 0·57 s.e. 0·02; standing: 0·42 s.e. 0·02).

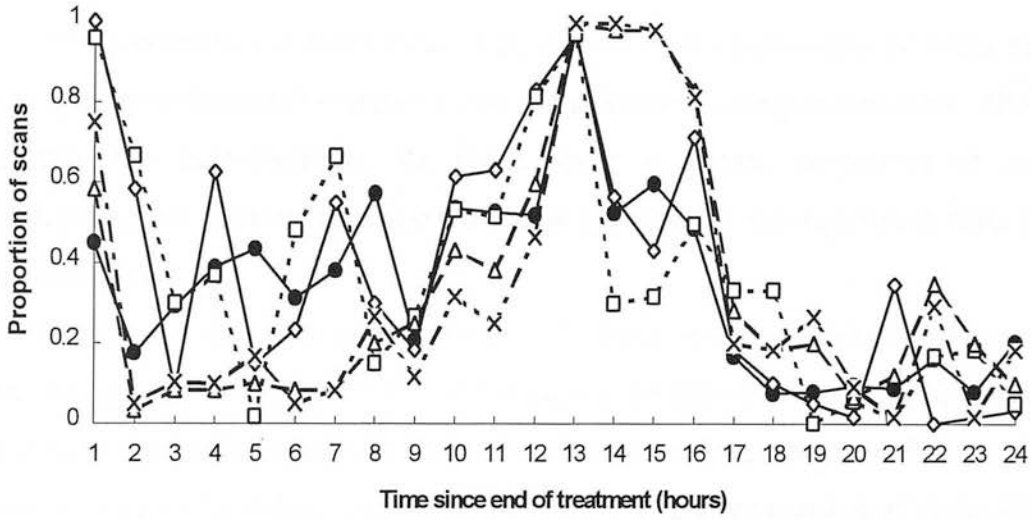

Figure 6.7. Effect of 15h transport and 12h food withdrawal post-treatment on the mean proportion of scans spent standing post-treatment compared with the equivalent time pre-treatment (n=6). For legends see Figure 6.4.

During the second 12h post-treatment F2 sheep spent a lower proportion of scans lying and a greater proportion of scans standing than at the equivalent time pre-treatment (Table III). F2 sheep spent a lower proportion of scans lying than F1 sheep and a greater proportion of scans standing than F1 sheep during the second 12h post-treatment (Table III). For F1 sheep, there was no effect of time on the proportion of scans spent standing and lying during the second 12h post-treatment.

During the first 12h post-treatment, sheep got up from a lying position more often (12h median/sheep: 16·5, Q1 13·5, Q3 19) than during the equivalent 12h pre-treatment (12h median/sheep 13, Q1 9·5, Q3 15). During the second 12h post-treatment period, sheep got up from a lying position less often (12h median/sheep

14, Q1 12, Q3 17) than during the equivalent 12h pre-treatment (12h median/sheep 17, Q1 13·5, Q3 20).

F2 sheep got up from a lying position more often than F1 sheep during the first 12h post-treatment (12h median/sheep: 1st 12h post-treatment: F1 14 (Q1 7·5, Q3 16·5), F2 17·5 (Q1 16·5, Q3 21)), and during the final 6h post-treatment (6h median/sheep: F1 7 (Q1 5·5, Q3 9), F2 9 (Q1 9, Q3 11).

Other behaviour

Post-treatment, F1 sheep showed no change in the proportion of scans spent investigating environment compared with the equivalent time pre-treatment. During the first 12h post-treatment, F2 sheep spent a greater proportion of scans investigating the environment than F1 sheep and than at the equivalent time pre-treatment (Table III).

During the first 12h post-treatment, F2 sheep spent a greater proportion of scans idling than F1 sheep and than at the equivalent time pre-treatment (Table III). After F2 sheep had been fed, there was no difference in the proportion of scans spent idling by F1 and F2 sheep. Control sheep spent a greater proportion of scans idling (0·44 s.e. 0·07) than at the equivalent time pre-treatment for the first 12h post-treatment (0·19 s.e. 0·02), but transported sheep showed little change from the proportion of scans spent idling pre-treatment (pre-treatment: 0·27 s.e. 0·03; post-treatment: 0·30 s.e. 0·07).

During the first 12h post-treatment, there was a higher occurrence of butting (12h median/sheep 5, Q1 3, Q3 7) than during the equivalent 12h pre-treatment (12h median/sheep 0, Q1 0 Q3 1). There was no effect of transport or feeding regime on the occurrence of butting.

6.4 Discussion

In this study, the sheep were returned to their home pens after transport. The commercial transportation of sheep involves exposure to novel environments which may have additional/overriding effects on the behavioural responses observed in this study. Grandin (1980) stated that unfamiliar environments are one of the most

stressful aspects of pre-slaughter handling and a number of studies have demonstrated that it can take weeks before sheep are adapted to new environments (McNatty *et al.*, 1972; Pearson & Mellor, 1976; Done-Currie *et al.*, 1984; Fordham *et al.*, 1991).

As said previously, care should be taken when interpreting the results of this study, as the group-housing of the sheep used for behavioural observations may have affected the independence of the behaviour of the individual sheep. Group-housing of sheep is preferable to the individual housing (even though the behaviour of each sheep would be independent), as individual penning is considered to adversely influence the behaviour of sheep (Done-Currie *et al.*, 1984; Marsden & Wood-Gush, 1986). An improvement of the experimental design would be to increase the number of groups used for each treatment. This would have greatly increase the cost of the study and the time required to perform it, which was not feasible. The effects of transport and the associated food deprivation on the behaviour of sheep in this experiment are consistent with those found by others. Confinement on a moving vehicle reduced the amount of lying and ruminating and increased the proportion of scans spent standing. This has been reported by a number of other authors (Ewbank & Kent, 1990; Cockram *et al.*, 1996). The post-treatment changes are also consistent with other work. The increase in feeding seen when given access to food has been reported following transport and food deprivations (Knowles *et al.*, 1995; Cockram *et al.*, 1996; Hall *et al.*, 1997; Sibbald, 1997) and field trials have reported sheep have an increased interest in feeding following transport (Knowles *et al.*, 1994; 1996). This consistency of the results from this experiment with previous work would suggest that despite the statistical non-independence of the individual experimental units used in the analyses, the results give a strong indication as to the effects of the treatments used.

The increase of time spent idling and the large increase in foraging behaviour seen towards the end of the first 12h post-treatment period suggest that the failure to provide food does influence the behaviour of the sheep in this study. As time without food increases, the sheep begin to actively seek food and become increasingly restless (in terms of the number of times sheep got up from a lying

position). The increase in time spent investigating the environment at this time and a pattern similar to foraging would suggest that this was also an expression of food-seeking behaviour, with both behaviours ceasing when F2 sheep were fed.

The increase in feeding behaviour seen immediately post-treatment, when F1 sheep were fed, and 12h post-treatment when F2 sheep were fed agrees with previous observations of sheep post-transport (Cockram *et al.*, 1996). The increase in feeding lasted for 4h for both feeding regimes, with no effect of transport, suggesting there was no difference in the effect of a 0h (CF1), a 12h (CF2) or a 27h (TF2) period of food deprivation on the motivation to feed. Alternatively, the lack of difference could result from a physical restriction on the amount of food that can be consumed.

6.4.1 Effect of transport

A journey of 15h has been shown to affect the behaviour of sheep being transported. The observed changes (increased standing, decreased lying and decreased rumination) have been reported in previous studies (Knowles *et al.*, 1993; Cockram *et al.*, 1996).

A surprising feature of the changes in the behaviour of transported sheep was that despite having no access to food, there was no difference in the proportion of scans spent eating during the final 8h of the journey compared with the non-transported sheep. The timing of the treatment is likely to be the cause of this, with feeding bouts in sheep at pasture being observed to cease between 22.00h and 07.00h (Lynch *et al.*, 1992). This time period corresponds to the final 8h of the journey in this study. This may suggest that scheduling journeys to occur overnight may decrease the overall impact of the food deprivation that occurs as part of the transportation process, and also provide added benefits through quieter roads and cooler temperatures. However, many journeys may require longer than 9h to reach their destinations. It must also be noted that whilst there was no difference between control sheep and transported sheep over the final 8h of the journey, there was a decrease in the proportion of scans spent eating during the whole journey for transported sheep when compared with the equivalent time pre-treatment. The lack

of difference during the second half of the journey may therefore be due to the decreased synchronisation of behaviour between animals that can occur in housed ruminants (Miller & Wood-Gush, 1991).

The changes observed in the control sheep during the first few hours of the treatment period may be explained by the fact that immediately prior to the start of the treatment period all behaviour sheep were removed from their pens to be weighed. This, and the loading of neighbouring groups onto the vehicle, may have caused a disturbance affecting their behaviour, particularly causing an increase in standing. Fresh food was also provided at this point, which caused an increase in feeding behaviour and consequently increased the proportion of scans spent standing.

The decrease in the proportion of scans spent ruminating during transport agrees with previous work on transported sheep (Knowles *et al.,* 1993; Cockram *et al.,* 1996), and along with changes in standing and lying, it would indicate that confinement in a moving vehicle inhibits/restricts some behaviours. Cockram *et al.* (1996) found that space allowance during transport affected the amount of lying and ruminating, with decreased amounts observed at lower space allowances of 0·22m^2/sheep (similar to the space allowance used in this study). As it has been suggested that rumination is inhibited in adverse situations (Beilharz, 1985), the restriction of lying and the close proximity of other sheep in this study may have inhibited the occurrence of rumination during transport.

6.4.2 Post-treatment recovery

The lack of difference between groups in the amount of time spent feeding post-treatment after being fed may indicate that this is not an appropriate measure of changes in feeding behaviour in response to food deprivation. Sibbald (1997) suggested that food restriction affected food intake through changes in meal frequency and rate of feeding, reporting higher intake rates for sheep with limited access to feed (6h) than for sheep with *ad-libitum* access to feed. Changes in the time spent feeding were reported in response to body condition (Sibbald, 1997). As the hay intake in this study was only measured over 12h periods, any changes in intake during the first few hours will not have been apparent.

The increase in feeding behaviour in F2 sheep after the provision of food at 12h post-treatment was also seen in F1 sheep, although of shorter duration. This may suggest the sheep were still recovering from the 15h food deprivation during transport by increasing their food intake. However, at the equivalent time pre-treatment, all groups showed a similar increase in feeding behaviour. This corresponded to the time when fresh food was provided. As food was still present in the pens at this time, it would suggest feeding is stimulated by the provision of fresh food. This has been reported by others working with sheep (Sibbald, 1997), and cattle (Winter & Hillerton, 1995).

The increased liveweights of F1 sheep at 12h post-treatment were due to the hay intake and the larger water intake during the first 12h post-treatment, previous studies have reported that most weight loss that occurs during the first 24h of food deprivation is due to rumen fluid loss (Hecker *et al.*, 1964).

The larger volume of water consumed during the first 12h post-treatment by F1 sheep would be a direct consequence of the increased feeding during this period. There was no observed increase in the frequency of drinking during the first 12h, so the intake of water during each bout of drinking must have increased. Saliva production increases in response to feeding, contributing to the increase in the water requirement of feeding sheep, with an estimated 2·7ml saliva being swallowed with every 1g dry food consumed (Stacy & Warner, 1966). The low water intakes of F2 sheep during the first 12h post-treatment agree with previous studies showing that sheep deprived of food drink little water (Hecker *et al.*, 1964).

Higher mean intakes of both hay and water were recorded in the group pens (pre-treatment: hay-1·1kg; water-3·3 litres) than in the individual pens (pre-treatment: hay- 0·8kg; water- 2·0 litres). A probable cause of this is social facilitation (Tribe, 1950), previous work has shown that this is responsible for initiating meals in sheep (Rook & Penning, 1991). This highlights a potential problem with the use of individually housed sheep in experiments, as there is a large amount of synchronisation of behaviour in sheep, and housing them separately may affect the results obtained.

During the first 12h post-treatment, F2 sheep spent more time lying than F1 sheep and than at the equivalent time pre-treatment, which may suggest increased resting, supported by the fact that the increase in lying behaviour in F2 sheep that were transported lasted longer than the increase in F2 sheep that were not transported. This may suggest that being transported for 15h at a space allowance of $0{\cdot}2m^2$/sheep was tiring. Increased lying behaviour post-transport was not seen after a 12h journey (Cockram *et. al.*, 1996). As for F1 sheep in this study, those sheep had access to food after being unloaded. This supports the suggestion that post-transport, sheep are primarily interested in feeding, with lying occurring only after sheep have fed or when food is not available.

The amount of rumination by F2 sheep was lower during the first 12h post-treatment than during the equivalent 12h pre-treatment. As rumination follows periods of feeding (Lynch *et al.*, 1992), the absence of food may limit the rumination that can occur.

The increase in butting seen over the first 12h post-treatment would suggest an increase in aggression (Hafez *et al.*, 1969). The causes of this may be different for the two feeding treatments. For F1 sheep, while sufficient space was provided for all sheep to feed at once, the inter-individual distances were greatly reduced during feeding. This increases the chance of disturbance of an individual's 'personal space', which will increase the likelihood of confrontation as animals attempt to preserve this space (Fraser & Broom, 1990; Murphy, 1990). F2 sheep may have become increasingly frustrated during the 12h period as the food-seeking behaviour did not achieve the intended goal. This may have increased aggression, as has been shown in other species (Duncan & Wood-Gush, 1971).

6.5 Conclusions

Withholding food following transport affects the behaviour of sheep, increasing the amount of food-seeking behaviour. This may cause 'stress', as the sheep are prevented from achieving a goal. Once food is provided, the additional period of deprivation had little effect on the observed changes in behaviour. These results suggest that discussions about the effects of food deprivation should perhaps

be more focused on the effect of denying the sheep the opportunity to perform the behaviours related to feeding, rather than the effect of different lengths of deprivation, as increasing the length of food deprivation from 15h to 27h had no apparent effect on the changes in behaviour seen in this study.

Post-treatment food-withdrawal did not effect the speed of recovery (in terms of returning to the pre-treatment pattern of behaviour), with both F1 and F2 sheep taking similar periods of time (15-17h) to return to pre-treatment levels and following a similar pattern of changes after being provided with food.

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CHAPTER 7 The effect of food deprivations of up to 24h on the motivation of sheep to feed.

Abstract

The effects of the food restriction that occurs during transportation on the welfare of sheep is as yet unclear. An operant crate and a push-door were used to measure feeding motivation of sheep after 0h, 6h, 12h, 18h and 24h without food.

In experiment 1 sheep had to push a panel with their nose to obtain a food reward. In experiment 2, they had to run a race and push through a weighted door to reach food. The time taken to reach various points was recorded and the work done to push through the door was calculated. In experiment 3, the sheep had to run a race and push through a weighted door as for experiment 2, but on reaching the reward pen, sheep were allowed to feed for 10 minutes. They were then returned to a holding pen and re-presented with the push-door. This was repeated until the sheep refused to go through the door. The frequency of passes through the door and time spent feeding in the reward pen were recorded, and amount of hay consumed was measured.

In experiment 1, three out of 12 sheep were trained to push the panel and the sheep differed in the mean number of times each would push the panel to obtain a food reward. There was no effect of treatment on the number of panel presses performed. In experiment 2, 10 out of 14 sheep became trained to use the push-door, and in experiment 3, 12 out of 14 sheep were successfully trained to use the push-door.

Sheep went through the push-door more frequently when food deprived, and they were quicker to enter the race (2·54s), reach the push-door (3·65s), and reach the food (7·70s) than when they had not been food deprived (time to enter race:

16·71s; time to reach push-door: 14·25s; time to reach food: 25·83). They also spent less time pushing the door (1·56s) than non-deprived sheep (3·31s). Food deprived sheep went through the push-door more times (8), consumed more hay (0·75kg) and spent longer feeding (2793s) than non-deprived sheep (times through door: 2; hay intake: 0·07kg; time feeding: 337s).

The push-door was considered to be a more appropriate method of measuring motivation to feed in sheep, as more sheep learned the task, and in less time than for the push-panel technique. As measured by the time taken to enter the race, reach the push-door, push through the door and reach the food there was an increase in feeding motivation after only 6 hours without food, but this motivation was reduced after the sheep had fed for 60 minutes.

7.1 Introduction

Sheep are selective grazers, spending 9-11h/day grazing when at pasture (Hafez *et al.*, 1969). They can be exposed to long periods of food deprivation during transport and when held at markets and slaughterhouses. After a 15h journey by road, sheep spent an increased amount of time feeding during the first 12h after unloading compared with the equivalent period pre-transport (Cockram *et al.*, 1996). This would suggest that feeding has a high priority after a period of food deprivation and transport, but little is known about the motivation of sheep to feed after such periods of food deprivation.

Dumont & Petit (1995) exposed sheep to 24h and 16h fasts and found that after fasting sheep would consume larger quantities of a readily available poor quality hay with no effect on the intake of a higher quality hay for which they had to work (by walking around an arena). This demonstrates an effect of food deprivation on food selection, increasing the acceptability of lower quality forage which may suggest an increased motivation to feed.

Operant conditioning has been used to investigate feeding motivation in a number of species, using the level of response as a measure of the feeding motivation. Examples can be found for a number of species including pigs

(Lawrence *et al.*, 1988; 1989; Lawrence & Illius, 1989), rats (Miller, 1955; Teitelbaum, 1966), and poultry (Duncan *et al.*, 1970; Duncan & Hughes, 1972). Other methods include aversion tests, for example using food adulterated with increasing concentrations of quinine. In rats, increasing the length of food deprivation was found to increase the concentration of quinine that would be tolerated in the food (Miller, 1955).

The measurement of motivation has become increasingly important in animal welfare as it attempts to provide objective measurements of subjective states. Three experiments using operant conditioning were performed to find an appropriate technique for measuring feeding motivation in sheep. This is a technique that has been used successfully with sheep, with a variety of required responses (Kilgour *et al.*, 1991). In experiment 1, the feeding motivation of sheep after food and/or water deprivations of up to 24h was examined, using the number of times that sheep would press a panel with their nose to obtain small amounts of whole oats as a measure of the motivation to feed.

The subsequent experiments used a push-door, a method that has been used with chickens to assess a number of motivational strengths (Duncan & Kite, 1987; Petherick & Rutter, 1990). The response required of the animals is primarily locomotion, and is one that is more associated with the foraging behaviour of sheep than that required in experiment 1. Experiment 2 examined the strength of feeding motivation in sheep after deprivation times from 6h to 24h, using time to pass through the push-door and the work performed in opening the push-door as a measure of their motivation to feed.

Experiment 3 investigated the way in which the motivation to reach food changed as food-deprived sheep were allowed to feed for short periods after working to reach the food reward.

7.2 Materials and Methods

7.2.1 Experiment 1- The feeding motivation of sheep after food and/or water deprivation

Animals and management

Twelve group-housed Suffolk ewes aged between two and four years were used. They had previously been gentled (see below) and were offered *ad-libitum* hay with concentrate supplement (250g/sheep of a commercial supplement fed at 07·00h). The sheep were trained to use an operant crate that had previously been used with rams (Blisset *et al.*, 1990), in which they had to push a panel with their nose to obtain a reward of 10g whole oats.

Gentling

The sheep were given 2h exposure to the handler every day for 3 weeks, with the handler sitting passively in the home pens. When a sheep approached the handler, a handful of whole oats were offered and as the sheep approached and fed more readily the handler gently stroked the sheep across the shoulders/back (the head area was avoided as this is thought to be the most guarded area of personal space). This continued until the sheep readily approached the handler when in the pen and did not react to being touched.

Training

Initial training allowed sheep to familiarise themselves with the equipment. They were placed in a deprivation pen with a non-experimental 'buddy' sheep (another sheep from the home pen, so the experimental sheep was not isolated) in the adjacent pen (Figure 7.1), and allowed to enter the crate as they wished. Pellets were fixed onto the panels to encourage the sheep to approach and investigate. The sheep were given 20 training sessions, each lasting up to 3h, over an eight week period. Sheep were trained automatically with no manual shaping, by allowing them free access to the crate which was left running on a training program, starting at a progressive ratio schedule (PR) of one and increasing by one after every 10 rewards.

This continued up to PR10. Once PR10 was reached, the sheep were observed as they worked the panels to ensure they were pushing the panels reliably (i.e. pushing the panel with the muzzle, not inadvertently hitting it). When they had reached this stage, they were considered to be fully trained.

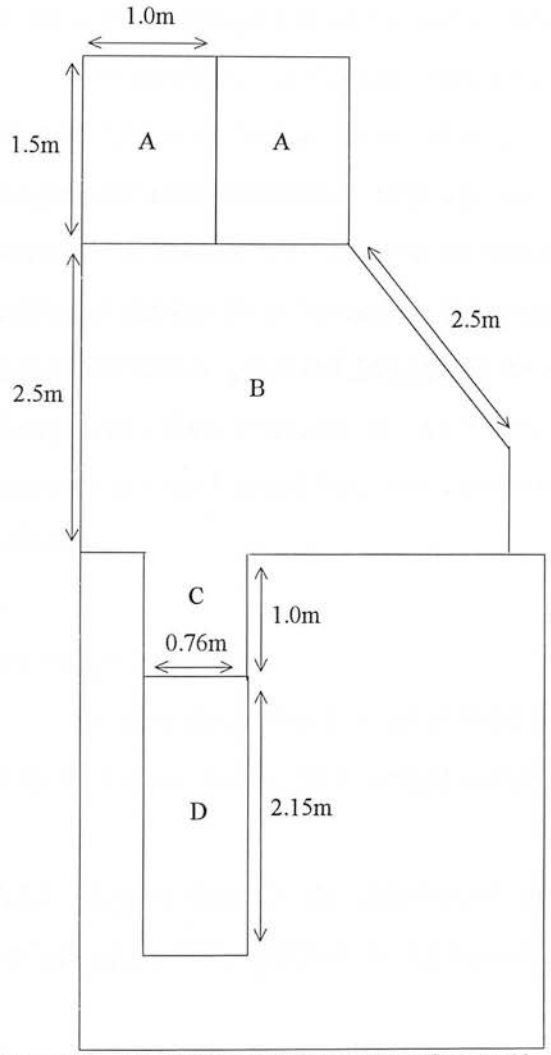


Figure 7.1. Plan of deprivation pens and crate (A- deprivation pens, B-test area, C-ramp, D-operant crate).

Procedure

At 08·00h on treatment days, the trained sheep were placed in a pen bedded with wood-shavings, with a 'buddy' sheep in an adjacent pen (Figure 7.1). Each sheep was exposed to four treatments (a control involving no deprivation of food or water (FW); food with no water (F); water with no food (W); no food and no water

(N)) at each of four deprivation lengths (6h, 12h, 18h and 24h) in a random order. The sheep were exposed to one treatment and one deprivation length each week. A 6-day interval was allowed between treatments to enable complete recovery from the period of food and water deprivation (Knowles *et al.*, 1993). The treatments began at the same time of day to ensure that the sheep were in a similar physiological state at the start of each period of food deprivation.

On completion of the deprivation period, the sheep were placed in the test area (Figure 7.1), with free access to the operant crate for 1h, with the crate running on a progressive ratio schedule, starting at one, and increasing by steps of two after each reward. A session was considered finished when there was an interval between pushes of greater than 2 minutes. On completion of testing, the sheep were returned to the deprivation pen until 24 hours from the start of the treatment had elapsed. The sheep were then returned to the home pen. This was performed so that no association was learned between completion of testing and being returned to the home pen.

Statistical analysis

As only three sheep were trained to use the equipment, the results will be described qualitatively, as the small sample makes the data unsuitable for analysis.

7.2.2 Experiment 2- An assessment of the feeding motivation of sheep after periods of food deprivation, using a push-door

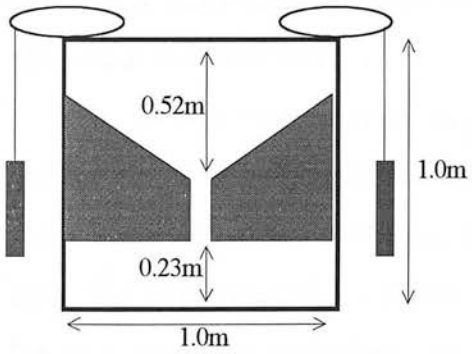
Animals and management

Fourteen 'gentled' ewes (see experiment 1), aged between two and four years and housed in pairs, were trained to use an unweighted push-door to reach hay (Figure 7.2). Eleven of the sheep had been used eight months previously in Experiment 1. All sheep were gentled using the same procedure as used for experiment 1, using concentrate pellets in place of whole oats. The sheep were then allocated to experimental pairs (with the restriction that they could not be paired with

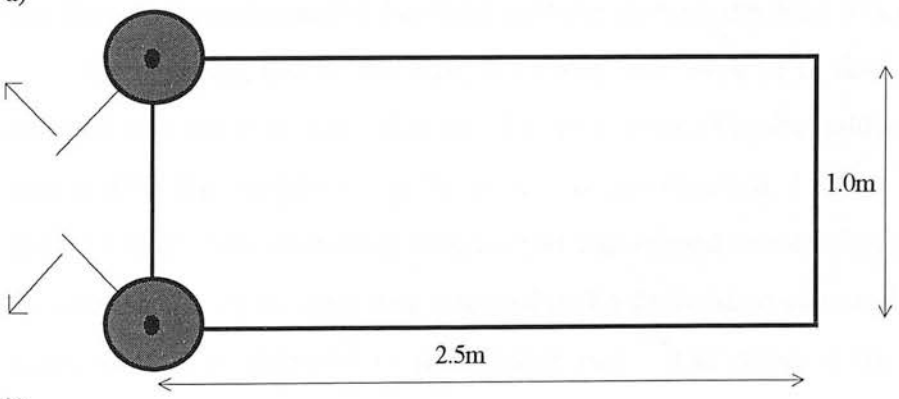
their pen-mate as this would be their buddy during testing, as it was the sheep most familiar to them).

Training

Training initially involved familiarising the sheep with the race layout (Figure 7.3). They were left in the holding pen (B) for 2h with continual access to the reward pen (E) through the race (C) and the open push-door (D). Hay was available in the reward pen, with water in the holding pen. Following this, the sheep were given training sessions consisting of 15 presentations at the door. The doors were gradually closed over the training period, each side of the door being closed by a quarter after five successful passes. This was continued until they had completed 10 consecutive passes through the closed push-door, when they were considered to be fully trained. Throughout training, the sheep's pen-mate was in the 'buddy' pen (F).



a)



b)

Figure 7.2. a) Push-door, b) aerial plan of push-door and race.

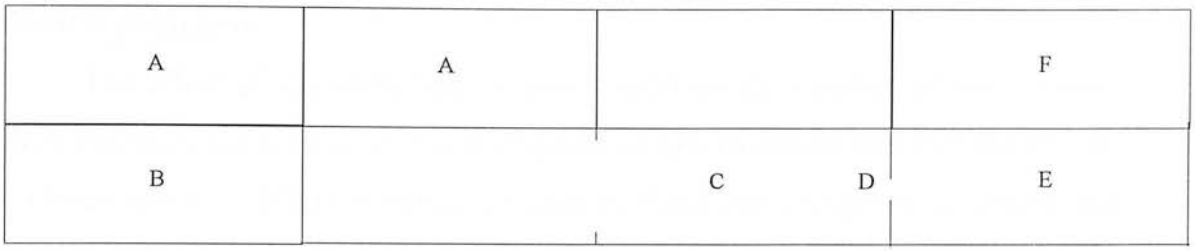


Figure 7.3. Plan of pen layout. A-Deprivation pens, B-Holding pen, C-Race, D-Push-door, E-Reward pen, F-'Buddy' pen (each pen measured 2·5m x 1m).

Procedure

Six treatments were used: control 1(no food deprivation with no food reward tested after 6h); control 2 (no food deprivation with a food reward tested after 24h); 6h food deprivation; 12h food deprivation; 18h food deprivation and 24h food deprivation. These were paired to allow two sheep to be tested each day with an interval of at least 12h between testings, to allow the sheep to settle after any disturbance caused by one of the pair being tested. Each pair was then exposed to the pairs of treatments in a random order. A 6-day interval between treatments was allowed as for experiment 1.

At 08·00h, experimental sheep were placed in the deprivation pens that were bedded with wood-shavings and contained water. On completion of a treatment, the sheep were moved to the holding pen and their pen-mate was moved to the 'buddy' pen. The experimental sheep was then released into the race. Time taken to enter the race, reach the push-door, open the push-door, reach the food and the distance the weights were moved by the sheep pushing through the door was recorded.

On reaching the reward pen, the sheep was allowed to feed from *ad-libitum* hay in a rack for 1 minute, after which it was returned to the holding pen. This was repeated for five weightings of the push-door (unweighted, +4·4kg; +8·8kg; +13·2kg and +17·6kg). The maximum weight used was around one-third of the liveweight of the sheep. The sheep were then returned to the deprivation pen until 24h had elapsed when they were returned to their home pen. The sheep were returned to the deprivation pens so that no association between the completion of testing and being returned to the home pen was learned. Whilst in the deprivation pens both control treatments had access to *ad-libitum* hay.

Statistical analysis

The effect of treatment (deprivation length) on the number of times sheep passed through the push-door was investigated using a Friedman two-way analysis of variance (n=10). Where significant effects of deprivation length were found, the critical differences for all pairs of treatments were determined (Siegel & Castellan, 1988).

The rate at which each sheep worked to push through the door was calculated for the treatments involving food deprivation, from the distance the weights were moved, the time spent pushing the doors and the added weight (Abbott, 1978). Analysis of the times taken to perform each procedure, and the work performed by the sheep to push through the door was made using a two-way analysis of variance (n=10), using a general linear models procedure as the experiment was unbalanced because one sheep did not undergo all treatments (Hatcher & Stepanski, 1994). The analysis examined the effects of treatment, and weight added to the push-door. Where significant effects were found, Tukey's Studentized Range (HSD) test was performed to examine differences between pairs of treatments. Due to the absence of work to obtain feed in most of the control sheep (few control sheep moved through the push-door), only the four deprivation treatments were examined in this analysis.

The relationship between the length of the food deprivation and the time to reach the race, the push-door and the food and the time spent pushing the door was investigated by using linear regression analysis. If data were skewed, the regression was conducted on transformed data (log10). The linear regression lines and their residual sum of squares were calculated for each behaviour regressed against the length of food deprivation (Ryan *et al.*, 1992). As a significant difference between non-deprived and deprived treatments was found using the Friedman two-way analysis of variance, the regression analysis did not include the control treatments.

7.2.3 Experiment 3- An assessment of the change in feeding motivation of sheep after food deprivations of up to 24h

Animals and management

Fourteen 'gentled' ewes (see experiment 1) used six months previously in experiment 2 and housed in the same pairs, were trained to use an unweighted push-door to reach hay (Figure 7.2). The sheep were fed *ad-libitum* hay and had constant access to water.

Procedure

Six treatments were used: control 1 (no food deprivation tested after 24h); control 2 (no food deprivation tested after 6h); 6h food deprivation; 12h food deprivation; 18h food deprivation and 24h food deprivation. As for experiment 2, these were paired to allow two sheep to be tested each day with an interval of at least 12h between testings, to allow the sheep to settle after any disturbance caused by one of the pair being tested. Each pair was then exposed to the pairs of treatments in a random order. A 6-day interval between treatments was allowed as for experiment 1.

At 08·00h, experimental sheep were placed in the deprivation pens that were bedded with wood-shavings and contained water. On completion of a treatment, the sheep were moved to the holding pen and their pen-mate was moved to the 'buddy' pen. The experimental sheep was then released into the race, with the push-door set at the highest weighting from experiment 2 (+17·6kg).

On reaching the reward pen, the sheep was allowed to feed from a known weight of hay (0·5kg) for 10 minutes (or until the sheep left the reward pen), after which the sheep was returned to the holding pen. Unconsumed hay was weighed and more was added if necessary. The hay was placed back in the pen and the sheep was re-released into the race. This was repeated until the sheep refused to go through the push-door within two minutes of release from the holding pen. The sheep were then returned to the deprivation pen until 24h had elapsed from the start of the treatment and then returned to their home pen as for experiment 2.

Statistical analysis

The effect of food deprivation on time spent feeding, and the total hay intake was analysed using a one-way analysis of variance (n=12). Where significant effects were found, Tukey's Studentized Range (HSD) test was performed to examine differences between pairs. The effect of food deprivation on the number of passes through the door by each sheep was analysed using the Friedman two-way analysis of variance (n=12). Where significant effects of deprivation length were found, the critical differences for all pairs of treatments were determined (Siegel & Castellan, 1988).

The relationship between the length of the food deprivation and the number of times sheep went through the push-door, the time spent feeding and the hay intake was investigated by using linear regression analysis. If data were skewed, the regression was conducted on transformed data (log10). The linear regression lines and their residual sum of squares were calculated for each behaviour regressed against the length of food deprivation (Ryan *et al.*, 1992). As a significant difference between non-deprived and deprived treatments was found using the Friedman two-way analysis of variance, the regression analysis did not include the control treatments.

7.3 Results

7.3.1 Experiment 1

Only three out of the 12 sheep became fully trained. Figure 7.4 shows the mean number of rewards/session for all treatments for the three sheep that were trained. There were no obvious differences between treatments, with no effect of length of deprivation on the mean number of rewards obtained/session (mean 6h: 19·5, 12h: 21·3, 18h: 20·5, 24h: 20·5). There was a large difference in the mean number of rewards obtained/session by each sheep (sheep1 29·81, s.e. 1·36; sheep2 19·56, s.e. 1·00; sheep3 11·69, s.e. 0·79).

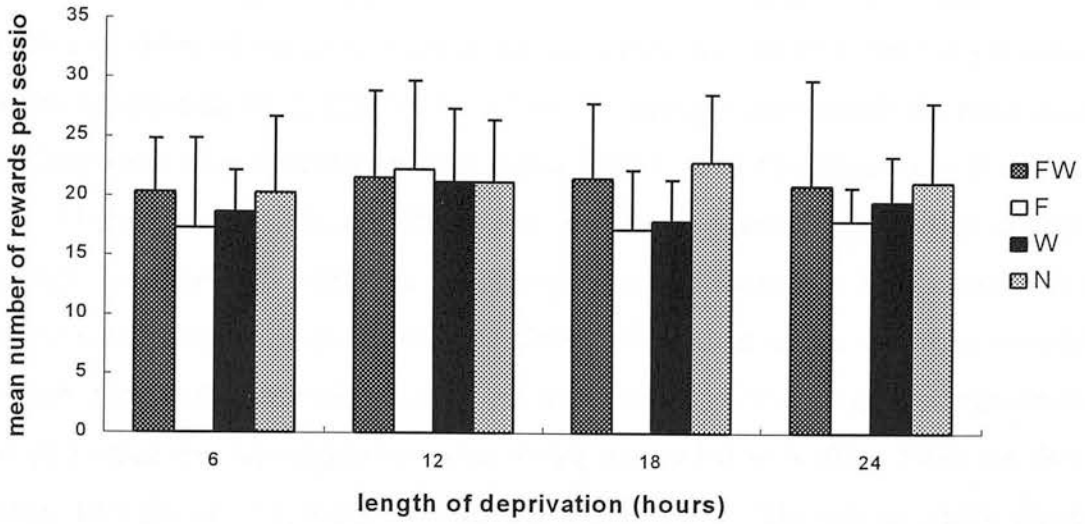


Figure 7.4. Mean number of rewards per session obtained by the sheep using the operant crate in Experiment 1 after periods of food deprivation (n=3). FW- food and water; F- food no water; W- water no food; N-no food no water. Vertical bars show s.e.

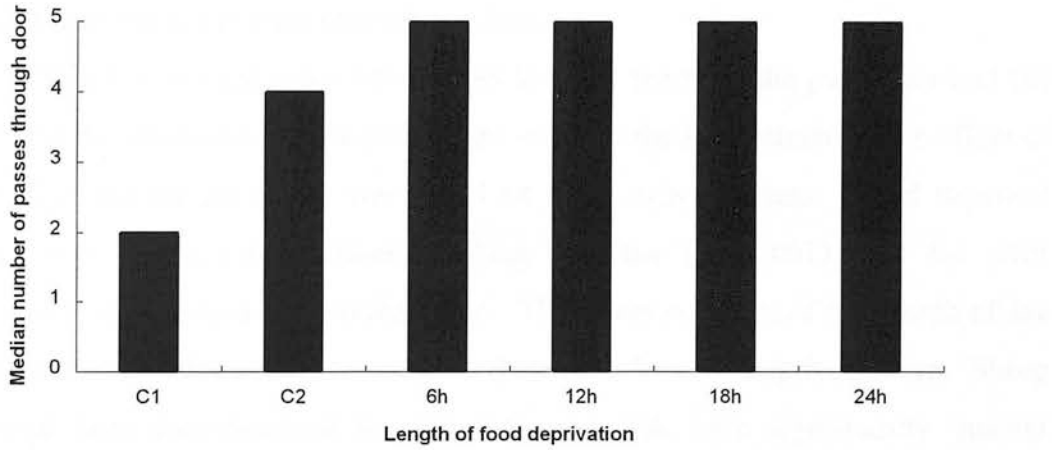


Figure 7.5. Median number of times sheep successfully passed through the push-door for each treatment (n=10). C1-control 1; C2-control 2; 6h-6h food deprivation; 12h-12h food deprivation; 18h-18h food deprivation; 24h-24h food deprivation.

7.3.2 Experiment 2

Ten out of 14 sheep became fully trained, two of which had not been used in experiment 1. The sheep took an average of 4·5 sessions (minimum 2, maximum 8) over a three week period to become fully trained. Figure 7.5 shows the median number of times sheep passed through the door for each treatment. Sheep went through the push-door less frequently after the two control treatments than after

treatments involving food deprivation ($p<0.001$). There was no difference in the number of times sheep went through the push-door for the four food deprivation treatments (median 6h: 5 12h: 5 18h: 5 24h: 5). Sheep went through the push-door less frequently after control 1 (median 2) than after control 2 (median 4) ($p<0.001$).

There was a significant difference in the rate at which sheep worked to push through the door at the different weightings, with the mean rate increasing as the weight on the push-door increased ($p<0.0001$). The rate at which the sheep worked to push through the door when 8·8kg was added was significantly greater than when the push-door was unweighted or when 4·4kg was added to it (0kg: 4·4J/s s.e. 0·9; 4·4kg: 19·9 J/s s.e. 1·1; 8·8kg: 42·3J/s s.e. 5·8, $p<0.05$). The rate at which sheep worked to push through the door when 8·8kg was added to it was significantly less than when 13·2kg and 17·6kg were added to it (13·2kg: 59·9J/s s.e. 4·6; 17·6kg: 70·6J/s s.e. 5·0, $p<0.05$). There was no effect of the length of deprivation, and no interaction between deprivation length and weight on the push-door on the rate at which sheep worked to push through the door.

Table I shows the mean times taken to reach the race, the push-door and the food, and the mean times spent pushing the door for the six treatments. No effect of the weight on the push-door was found on these measurements. Food deprived sheep were significantly quicker reaching the race ($p<0.0001$), and the door ($p<0.0001$) than non-food deprived sheep. There was no effect of the length of the deprivation on the time taken to complete these tasks by food-deprived sheep. Sheep that had been food-deprived for 12h, 18h and 24h were significantly quicker reaching the food than non-deprived sheep ($p<0.0001$). Sheep that had been food-deprived for 6h were significantly quicker reaching the food than after the control 2 treatment ($p<0.0001$).

Food deprived sheep also spent less time pushing the door than non-food deprived sheep ($p<0.0001$). For the non-food deprived treatments, sheep were quicker to enter the race when there was food in the reward pen (control 2) than when there was no food in the reward pen (control 1) ($p<0.05$).

Linear regression analysis found a significant association between the length of the food deprivation and the time taken to reach the race, the push-door and the food and the time spent pushing the door (Table II).

Table I. Mean times (s.e.) taken by the sheep in Experiment 2 to reach race, push-door and food, mean time spent pushing the door and the mean rates at which food-deprived sheep worked to push through the door (n=10).

	Time to race (s)	Time to door (s)	Time to food (s)	Time pushing (s)	Rate of work (j/s)
No food deprivation					
control 1	21·77^{a} (4·46)	12·12^{a} (2·63)	21·49^{ab} (6·34)	3·40^{a} (0·44)	-
control 2	11·65^{b} (2·82)	16·38^{a} (3·96)	30·17^{b} (6·25)	3·21^{a} (0·46)	-
Length of deprivation					
6h	2·87^{c} (0·17)	4·00^{b} (0·18)	12·17^{ac} (2·94)	1·69^{b} (0·07)	36·47(4·44)
12h	2·54^{c} (0·17)	3·52^{b} (0·15)	5·56^{c} (0·22)	1·41^{b} (0·07)	42·42 (5·10)
18h	2·49^{c} (0·12)	3·56^{b} (0·14)	6·20^{c} (0·29)	1·54^{b} (0·10)	38·62 (4·61)
24h	2·41^{c} (0·08)	3·53^{b} (0·10)	6·86^{c} (1·37)	1·58^{b} (0·18)	42·50 (5·86)

a,b,c. Means in the same column with different superscripts are significantly different ($p<0.05$)

Table II. Results of linear regression analysis on length of food deprivation and times taken by the sheep in Experiment 2 to reach race, push-door and food, and time spent pushing the door (n=10).

	a	b	R^2	t	p
Time to race	3·23	-0·145	2·8	-2·34	0·021
Time to push-door	4·29	-0·141	2·5	-2·19	0·03
Time to food	14·7	-1·52	2·1	-1·99	0·048
Time pushing door	1·88	-0·0823	3·2	-2·48	0·014

a=intercept (y=a+bx)
b=regression coefficient or slope
t=b/SE(b)
R^2=square of correlation coefficient
p=significance of the regression coefficient

7.3.3 Experiment 3

12 out of the 14 sheep were fully trained, two of which had not learnt the task for experiment 2. Food deprived sheep went through the push-door more times (7-10) than non-deprived sheep (1-3) (Figure 7.6) ($p<0·0001$). They also consumed more hay (0·6-0·8kg compared with 0·04-0·1kg) ($p<0·0001$), and spent longer

feeding (35-57 minutes compared with 3-8 minutes) than non-deprived sheep ($p<0{\cdot}0001$). There was no effect of deprivation length on any of these measurements (Table III).

Linear regression analysis found a significant association between the length of the food deprivation and the number of times sheep would pass through the push-door ($p<0{\cdot}05$). There was also a significant association between the length of food deprivation and the time spent feeding ($p<0{\cdot}01$), but no association with the hay intake (Table IV).

Table III. Effect of food deprivation on the mean (s.e.) total weight of hay eaten and the time spent feeding in the reward pen (n=12).

	Hay intake (kg)	Time spent feeding (s)
No food deprivation		
Control 1	$0{\cdot}10^{a}$ (0·07)	485^{a} (370)
Control 2	$0{\cdot}04^{a}$ (0·01)	188^{a} (97)
Food deprivation (h)		
6	$0{\cdot}63^{b}$ (0·08)	2280^{b} (33)
12	$0{\cdot}67^{b}$ (0·10)	2121^{b} (291)
18	$0{\cdot}84^{b}$ (0·11)	3342^{b} (433)
24	$0{\cdot}84^{b}$ (0·07)	3428^{b} (340)

a,b,. Means in the same column with different superscripts are significantly different ($p<0{\cdot}05$).

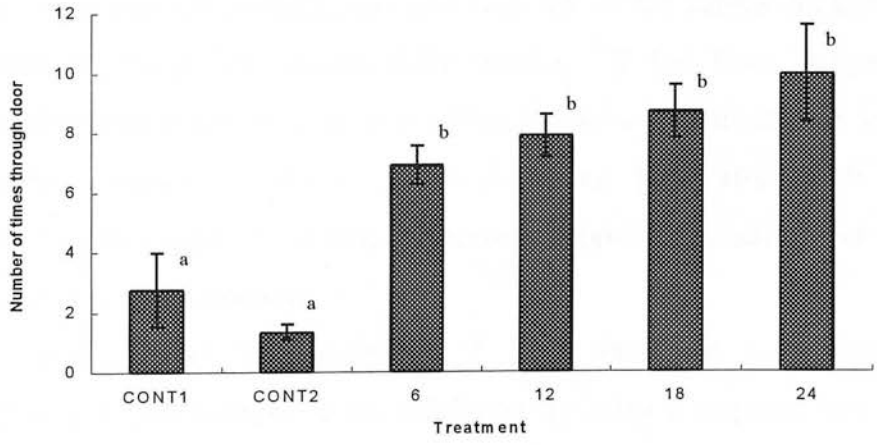


Figure 7.6. Mean number of times sheep passed through the push-door for each treatment (n=12). CONT1-no food deprivation tested after 24h; CONT2-no food deprivation tested after 6h; 6- 6h food deprivation; 12-12h food deprivation; 18-18h food deprivation; 24-24h food deprivation. a,b. Means with different superscripts differ significantly ($p<0{\cdot}0001$). Vertical bars show s.e.

Table IV. Results of linear regression analysis on length of food deprivation and number of times the sheep in Experiment 3 passed through the push-door, time spent feeding and hay intake (n=12).

	a	b	R^2	t	p
Times through door	3.88	1·00	10·9	2·3	0·027
Time spent feeding	674	469	16·3	2·9	0·006
Hay intake	0·371	0·0828	8·6	2·01	0·051

a=intercept (y=a+bx)
b=regression coefficient or slope
t=b/SE(b)
R^2=square of correlation coefficient
p=significance of the regression coefficient

7.4 Discussion

'Buddy' sheep were used in all experiments as isolation is known to be stressful to sheep (Parrott *et al.*, 1988), and was found to be a problem when training the sheep. In experiment 1, leaving the crate open, so the sheep could enter and leave as they wished presented the sheep with a choice: remain in visual and partial physical contact with another sheep or obtain food in the crate. In experiments 2 & 3, the buddy was penned next to the reward pen. Fewer sheep went through the push-door when there was no food reward (sheep could see into the reward pen over the push-door), which indicates that the sheep who pushed through the door were working for the food rather than the company of their pen-mate.

There were also problems attracting sheep to the panels in experiment 1. Attempts to increase the attractiveness and visibility of the panels did not increase the number of sheep that became fully trained. It has been suggested that incompatibilities between response and reinforcer make it difficult to train animals to make operant responses to obtain rewards (Dawkins, 1990; Breland & Breland, 1961), and this may explain the lack of success in training the sheep and the large individual variation in responses.

The method used in experiments 2 & 3 attempted to overcome this incompatibility between response and reinforcer by using a response more closely related to the feeding behaviour of sheep. More sheep learned the task over a shorter period of time (<1 month compared with 2 months), and while the three sheep

trained to use the crate were also trained to use the push-door, nine sheep were successfully trained to use the push-door that had not learned to use the crate. This increase in the ease of training may be a result of the sheep being more accustomed to both the handler and the surroundings, having been trained for the previous experiment. However, as two of the four sheep that had not been used in experiment 1 learnt to use the push-door, this would suggest that it was a more appropriate response than panel pressing.

Food deprivation resulted in an increase in the number of animals completing the task, and an increase in the speed at which they did so in experiment 2. In experiment 3, the sheep went through the push-door more often, consumed more hay and spent longer feeding when they had been deprived of food than when they had not been deprived. These results suggest that a greater motivation to feed exists after periods without food.

Increasing the length of the deprivation was found to have no effect on the mean times taken to enter the race, go through the push-door or reach the food in experiment 2. Although this experiment found no effect of length of food deprivation on these times, the significant associations between these factors would suggest that the length of time without access to food influenced the speed at which the sheep completed the task, as the length of the food deprivation increased, the sheep went through the push-door more quickly. This may indicate an increased motivation to reach the food in the reward pen. In experiment 3, whilst the increases in the mean number of times through the push-door were not significant, the significant association between this and the length of the food deprivation and between the length of food deprivation and the time spent feeding show that the longer sheep are without food (up to the 24h investigated in these experiments) the more time they will spend feeding and the more work they will do (in this case, the more often they will repeat a task to obtain limited access to food).

Petherick & Rutter (1990) found hens deprived for 12 and 43 hours spent the same amount of time pushing to gain access to food, but were quicker to get through the door after 43 hours food deprivation. The door used in that study differed from the one used in this study in that it was held shut by computer until a required force

was reached, then it was released. The authors felt this 'unnatural' behaviour of the door could have been mildly aversive to the hens. Whilst the forces exerted upon the push-door used in experiment 2 were less accurately calculated, it behaved more naturally, as it opened as the sheep pushed against it, and overcame the problems found by Duncan & Kite (1987) in their hen experiments, in that the force required by the sheep to push the door did not increase as the push-door opened due to the use of bearings.

Food-deprived sheep in experiment 3 were not motivated to obtain hay after they had fed for almost 1h in the reward pen. This would suggest that a period of 60 minutes may be sufficient to satisfy any 'need' that was experienced by the sheep as a result of the food deprivation treatments. There is likely to be an aspect of rumen capacity to the post-deprivation food intake. Whilst this alone does not control voluntary food intake, it may be a restricting factor in recovery from periods of food deprivation, as studies have shown intakes to be depressed when balloons of water or long fibres are added to the rumen (Forbes, 1995).

The consumer-demand theory of motivation put forward by Dawkins (1983) suggests that a demonstration of need requires the animal to be willing to experience some cost for the consummatory goal of its motivation. Lawrence *et al.* (1989) found the level of food restriction affected how willing pigs were to work for food. In experiments 2 & 3, the lower number of successful passes through the push-door in the control treatments compared with the food deprivation treatments would suggest that any reward obtained by passing through the push-door did not outweigh the cost of pushing against it. This is consistent with the consumer-demand theory of motivation, as when they were food deprived, sheep through the push-door more often.

7.5 Conclusions

These experiments have demonstrated that for measurement of feeding motivation, a traditional operant crate was not appropriate as only a small proportion of the population could be trained to use it. The push-door seems to be more

appropriate for sheep as the task was learnt in less time and by more individuals. The second experiment demonstrated an increase in the motivation of sheep to feed after just 6 hours without food. As transport of sheep may involve periods without food considerably longer than 6 hours, it is important that the welfare implications of this increased motivation are considered when formulating legal requirements for feeding intervals.

The third experiment confirms results of experiment 2. However, it is still not clear what effect this may have on their welfare. As the five freedoms of the Farm Animal Welfare Council include freedom from hunger (Farm Animal Welfare Council, 1994), work is required to establish whether an increase in motivation to feed in sheep is indicative of an experience that is comparable to the hunger felt by monogastrics. In all three experiments, sheep were familiar with the experimental conditions. Commercial transportation will involve sheep being fed (with food that may be novel) in novel environments. As sheep have been found to be neophobic regarding food in novel environments (Done-Currie *et al* 1984; Burritt & Provenza 1997), failure to provide sheep with food they will readily consume may not reduce the increased feeding motivation measured in this study, which may have implications for the welfare of sheep during and post-transport.

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CHAPTER 8 General Discussion

The work in this thesis aimed to investigate the behavioural responses of sheep to the food deprivation associated with transport. The response of sheep to food deprivations of 6h, 12h, 18h and 24h involved an increase in foraging behaviour. This was not seen when sheep were confined in unfamiliar pens or on vehicles for 15h and 24h. Following periods of food deprivation, the behavioural response of sheep was similar regardless of whether or not the sheep had been transported. An increase in feeding behaviour was observed, suggesting an increase in the motivation of sheep to feed following a period of food deprivation. The work described in chapter 7 attempted to quantify this increase in terms of the work sheep would do in order to gain access to food. Sheep were quicker to pass through the door to reach food, and did so more often after a period of food deprivation than when not food deprived, which would support the suggestion that the increased feeding behaviour is indicative of an increase in motivation to feed. However, the length of food deprivation had no effect on the measures of feeding motivation investigated during this thesis.

8.1 Response to food deprivation

Work described in chapter 2 demonstrated an increase in foraging behaviour and an increase in the number of steps taken during periods of food deprivation of up to 24h. This increase in foraging behaviour was also seen in chapter 6, where the effect of an additional period of food deprivation following a 15h journey was investigated. This increase was only observed during the additional 12h food deprivation post-transport.

The difference in response to food deprivation, seen as differences in the behaviour of sheep when deprived on a vehicle compared with sheep deprived in

their home pen, would seem to be dependent on the location. When in their home pens, sheep showed signs of actively seeking food which may indicate a potential welfare problem if being unable to achieve their goal causes frustration. As these food-seeking behaviours are not seen when the sheep are on lorries, and no other behaviour that was considered to be indicative of food-seeking was observed, two possible explanations can be found for this difference. The unfamiliar location and the restricted space could be inhibiting their ability to perform their normal behavioural repertoire. Alternatively, as the sheep are somewhere where they have not received food and the vehicle is unlikely to smell very strongly of food, these behaviours may not be stimulated. In the home pens, the sheep would be likely to have some expectation of being fed (due to their experience of being fed in these pens). A trial where sheep were kept in familiar pens but the olfactory cues were removed may show whether olfactory stimuli were an important external stimulus for behavioural evidence of a motivation to find food. Moving to an unfamiliar pen may override any such display of food-seeking behaviour, so simply moving the sheep to a pen that has no external food cues may not answer this question. Another possibility would be to use anosmic sheep, however this does not overcome the problem of expectation of food.

Moving sheep to a novel environment has been shown to affect the behaviour of sheep, causing them to withdraw from the environment, spend less time observing and spend more time in non-alert behaviours (Done-Currie *et al.*, 1984). This may affect their responses to food deprivation. Chapter 5 demonstrated a reduction in feeding behaviour by sheep when they were moved to an unfamiliar environment. It is possible that when moved to an unfamiliar environment the reaction to the new environment would override any food-seeking behaviour.

Another possibility for the increase in food-seeking behaviour seen in chapter 6 is that the sheep were penned in a large building containing other sheep that had access to food. Whilst the groups being deprived of food had been visually isolated, it is likely that they could still smell food in the building and hear other sheep feeding. Although work has shown that sheep do not commence or cease grazing at the same time (Arnold & Dudzinski, 1978), it has been suggested that social

facilitation operates to synchronise the start and end of grazing periods (Tribe, 1950), and this may have increased the desire of sheep in this trial to find food. This may also explain the increase in butting seen during this time (compared with chapter 2, where although it is possible that sheep could still smell food stored nearby, there were no sheep feeding in the shed). One of the main causes of aggression between ewes is competition for food (Lynch *et al.*, 1992), and work in other species (e.g. in poultry [Duncan & Wood-Gush, 1971]) has shown that frustration from not being able to gain access to food results in aggression. However, in stable groups (such as the ones used throughout this work), threats are more common than overt aggression, with display and intimidation being the most common forms of agonistic encounters rather than fighting (Lynch *et al.*, 1992). Threat behaviours can be subtle (Hafez *et al.*, 1969), which will make these behaviours more difficult to identify in a transport situation (compared with the more overt behaviours such as butting) and were not looked for during this work.

Future work could examine the occurrence of threats during and after periods of food deprivation and the outcome of such encounters, in particular, the occurrence of displacements from feeding places post-transport as this may influence the recovery of sheep from the food deprivation and journey, particularly if only a limited amount of food is provided.

Hall *et al.* (1997) reported that following a 14h confinement in a stationary trailer, a large number of displacements were observed at the feeding trough (more displacements occurring when hay was fed rather than concentrate). However, the number of displacements occurring before the period of food deprivation was not reported and so it is not clear if the large numbers are due to the period of food deprivation, the diets on offer or the management regime (e.g. space available at the feeding trough). If the sheep are not in a stable group having been mixed before/during transportation, there will be an increased probability of competitive social interactions, possibly resulting in injury. Work investigating the effect of group size on competition for feeding space found that the increased competition in larger groups resulted in sheep making fewer visits to the feeding stall (Jenkins & Leymaster, 1987). The sheep compensated for this by increasing the length of and

the amount consumed during each visit (Jenkins & Leymaster, 1987). The effect of food deprivation on the feeding pattern of these groups was not investigated. Whilst there was a reduction in the number of displacements occurring in the larger groups, the increased motivation to feed that will occur during periods of food deprivation may change this.

The work performed for this thesis would suggest that the lengths of deprivation investigated here do not greatly affect the responses of sheep to food deprivation (although a trend was seen in experiment 3 described in chapter 7 which suggests an increase in the number of times sheep would pass through the door to feed for a limited time as the length of the food deprivation increased.) More work with refined equipment or an alternative method of assessing motivation may provide stronger evidence of this trend.

If there is no effect of the length of the food deprivation (which for periods of up to 24h the work here would support) the question that follows is whether depriving sheep of food causes unnecessary suffering (rather than attempting to come up with a length of time that is acceptable). However, when you compare the behavioural responses with the biochemical responses, while there is little behavioural evidence of a response to the food deprivation during transport, free fatty acids and beta-hydroxybutyrate have been observed to increase. Knowles *et al.* (1993; 1996) found increased concentrations of free fatty acids after journeys of 9h, 14h, 15h and 24h compared with pre-transport concentrations. Knowles *et al.*, (1996) also found plasma concentrations beta-hydroxybutyrate increased significantly from pre-transport concentrations after 24h transport (Knowles *et al.*, 1996). Blood sampling was infrequent in both of these trials, only occurring at the end of the journeys in both trials. Cockram *et al.* (1997) took blood samples more frequently, every 3h during a 24h journey, and also reported increases in plasma concentrations of beta-hydroxybutyrate after 24h transport, with sheep transported for 24h having higher plasma concentrations than control sheep that were kept in their home pens with food and water. A point at which biochemical indicators of fasting increase significantly may be more important in deciding on suitable lengths of time for transport and lairage requirements. While such changes only indicate

that the sheep are responding to an energy deficiency caused by the food deprivation, they may provide a more quantitative measure of the response to food deprivation, and blood sampling is easier to do *in situ* than measuring the feeding motivation (by the methods currently available) to assess the response of sheep to a period of food deprivation. Ruminants possess a large reservoir of water and metabolites in the rumen which will enable them to tolerate periods of food and water deprivation more successfully than monogastrics. However, work in this thesis found evidence for an increased motivation to feed after only 6h food deprivation (increased feeding behaviour in chapter 2, and reduction of the time taken to reach a food reward, and an increase in the number of times sheep would pass through the push-door in chapter 7). Work has not shown a significant increase in the biochemical indicators discussed above after 6h of food deprivation. Other factors involved in the control of food intake may be more important than changing levels of blood metabolites after relatively short periods of food deprivation. This may also be evidence for an emotional element of feeding motivation that is similar to the human experience of hunger. Work in humans has found an increase in the urge to eat in the 2h before a meal, with changes in the mood of those questioned from positive to negative as the level of hunger increased (Monello & Mayer, 1967). This led the authors to suggest that 'ordinary' hunger (that experienced within the context of normal North American food intake, rather than starvation situations) was as much a cerebral experience as a physical experience. The problem with hunger as a welfare criteria, is that there are a diversity of definitions for it, ranging from an increased drive for food (Lepkovsky, 1973), to 'a state resulting from deficits which manifests itself in a disagreeable complex of sensations' (Janowitz, 1958). As it has been argued that welfare is purely a question of how an animal 'feels' (Duncan & Petherick, 1989), the sensation of hunger as well as the physical response to food deprivation is important in determining the effect of periods of food deprivation on the welfare of sheep. Use of techniques such as the push-door are an attempt to find objective methods of measuring motivation, as they may provide information as to the way animals experience subjective feelings (Petherick & Rutter, 1990).

An additional 12h food deprivation following transport (chapter 6) did not appear to increase the time taken to recover from transport and/or food deprivation. However, the post-treatment recovery was only followed for 12h in the groups given the additional period of food deprivation post-treatment. The behaviour post-treatment in the previous chapters returned to pre-treatment levels following the initial differences and showed little further change. This would suggest that the 12h observation period was sufficient to observe behavioural recovery from the treatments. The results from this work would appear to be in agreement with those of chapter 7, in that the motivation to feed is increased following periods of food deprivation, in terms of increased time spent feeding and a reduction in the time taken to use the push-door. The lengths of food deprivation used in this thesis did not have any additional effect on the motivation to feed. It would therefore appear that the response of sheep to a 6h food deprivation is the same as to a 24h food deprivation. In other parts of the world there are examples of journey times greater than the 24h maximum investigated during this work (van den Heever *et al.*, 1967; Yeh *et al.*, 1978; Wythes *et al.*, 1980; 1981). The effect of these greater deprivations on the indices used here would need to be investigated before any comment on the effects of periods of food deprivation greater than 24h could be made.

While experiment 2 in chapter 7 showed no difference in the motivation to feed for 6h, 12h, 18h or 24h food deprivations, experiment 3 would seem to indicate that for food deprivations of 6h, 12h and 18h there was a gradual increase in the amount of food/time feeding needed to reduce the motivation to reach food, although the results were not statistically significant. Lankin (1997) investigated the influence of feeding motivation on the behaviour of sheep, in terms of their withdrawal response from someone who after placing food in a trough, stood behind the trough for 12 minutes and touched the sheep that were feeding on the head and back with a paintbrush stained with a dye. The author found the length of the deprivation (from 1h up to 14h) affected the behavioural response of sheep, with the longer lengths of deprivation resulting in a reduction in the number of withdrawal reactions which the author put down to an increase in the feeding motivation. This would suggest the trend in the results of chapter 7 may be indicative of a gradual increase in feeding

motivation that reaches a plateau at 18h. If so, the length of lairage periods provided during transport could vary according to the length of the total journey, but a decision has to be made as to at what point the increase in motivation to feed constitutes a welfare problem that must be addressed by providing food during the journey. The results found here are a step on the way to making such a judgement, but appropriate methods of assessing welfare problems in such short-term situations are still being developed. While the lack of food may cause 'hunger', and the response of sheep unloaded into pens containing food would encourage this assumption, we do not yet know if this is the case, or how distressing such a sensation will be to an animal that has a rumen containing a large supply of potential energy resources. It is unlikely that a single method of assessment will be sufficient, instead using biochemical changes in combination with behavioural changes and work assessing the changes in motivation associated with these changes will give the best idea as to the impact they have on the welfare of an animal.

Once sheep begin to feed after a period of food deprivation, there is evidence of a redistribution of water, with an increase in PCV (chapter 3). Previous work has suggested that when sheep feed, large volumes of fluid pass across the rumen wall during eating, with 1·0-1·5l fluid entering the gut from the extracellular space (Ternouth, 1968; Christopherson & Webster, 1972). As a large increase in feeding was observed post-treatment in all experiments (apart from chapter 6), there is the potential for sheep to become dehydrated when fed hay after a period of food deprivation. The results of chapter 4 (which demonstrate that sheep eat before they drink during a 3h lairage period), combined with the results from chapter 3 (showing increases in both plasma vasopressin and plasma osmolality during the first few hours post-treatment when they spent a large proportion of time feeding) would suggest a high risk of sheep given a 3h mid-journey lairage period being dehydrated during the second part of the journey. The new transport regulations (The Welfare of Animals (Transport) Order, 1997) require sheep to have 1h access to water (and food if necessary) after 14h of transport in an appropriate vehicle. Chapters 2 and 3 demonstrate that sheep drink little water during periods of food deprivation (at

temperatures of up to 21°C). Work on sheep transported by rail for 2-5 days in South Africa (with temperatures greater than those experienced in the UK) found that following 2/3 day journeys, sheep showed no preference for either food or water. After a 5 day journey, sheep demonstrated a preference for water, feeding only after they had drunk (Sutton & van den Heever, 1968). This demonstrates that sheep will drink before feeding (suggesting thirst), but the length of water deprivation required to increase the motivation to drink above the motivation to feed is far greater than will generally be experienced during transport in the UK. Therefore, the provision of water alone during breaks in journeys is unlikely to result in sheep drinking.

If food is also provided, the results found during this work would suggest that the sheep will spend most of the time feeding, rather than drinking. Chapter 4 demonstrates that 3h may provide sufficient time for sheep to feed but not to drink, especially if the animals are kept on the vehicles at low space allowances. This will greatly increase the risk of dehydration during the remainder of the journey.

As sheep feed before they drink, limiting the food available to the animals during lairage could reduce the latency to drink. Work by Jenkins & Leymaster (1987) demonstrated that when held in larger groups (i.e. at lower space allowances), rams had a lower number of visits to the feeding trough, but when they were there, the length of the visit and the amount of food consumed increased. These results would suggest that reducing the amount of food available to sheep during a break in the journey would probably result in the food being consumed by the sheep that reach it first, which will not solve the problem of post-feeding dehydration for those sheep that do eat.

Changes in the water source and method of presentation may also contribute to the increased risk of dehydration. Chapple *et al.* (1987) reported a neophobia to novel containers that has also been reported in rats (Mitchell *et al.*, 1973). Knowles *et al.* (1993) reported that lambs would not readily drink from an unfamiliar source after deprivations of up to 24h. This may present a more serious problem for sheep that have previously drunk from natural supplies (streams etc.) than for those that have previously been given water from containers.

This is one situation where the change in environment may be beneficial. Unfamiliar environments resulted in less feeding (chapter 5), and a lower food consumption may reduce the risk of dehydration during the remainder of the journey. The lower feeding found in chapter 5 could be a result of the change in diet that accompanied the change in environment. Burritt & Provenza (1997) found that sheep were more neophobic at an unfamiliar location, but when fed a familiar food their intake was the same as when at a familiar location. For post-transport recovery, it would therefore be recommended that sheep are fed a food as familiar as possible to reduce the time taken to recover from the period of food deprivation as much as possible.

One point concerning the results from chapter 4 is that the lairage was in a familiar environment with a familiar food. The unfamiliar environment of the vehicle may partly explain the lower proportion of scans spent feeding (although the food was familiar). The effect of the familiarity of the food and environment are likely to affect the benefits of providing sheep with a period of lairage, if the aim of providing this break is to provide sheep with the opportunity to feed and drink. If the aim of providing the break is to facilitate resting, the decreased eating is associated with an increase in lying (and therefore resting), but the unfamiliar environment increases the length of time taken to return to 'normal' levels of behaviour (if the levels of behaviour observed pre-treatment are 'normal').

Following periods of food deprivation (regardless of whether the sheep were transported), similar increases in feeding behaviour were seen in all trials apart from when sheep were moved to a novel environment. This increase would give some evidence for the presence of some kind of a 'need' for food following periods of food deprivation lasting between 6h and 24h. Chapter 7 attempted to find a method of quantifying this 'need' and to investigate how long they would continue to work for food rather than just using their response to *ad-libitum* access to food (the only measure of motivation available in chapters 2, 3, 4, 5 and 6). The results show that the sheep used in the trial would work for around 60 minutes access to food, consuming around 1kg of hay before they became unwilling to push through the door. The provision of this amount of hay (or equivalent of an alternative foodstuff)

post-treatment may be sufficient to reduce feeding motivation. However, the design of the trial may have caused an early reduction in the responses, through boredom or frustration as they were removed from the reward pen 10 minutes after they had passed through the door. The 10 minute feeding period chosen was perhaps too short a feeding period, feeding bouts for sheep at pasture last between 20 and 90 minutes (Lynch *et al.,* 1992). However, fresh food was provided for each run through the door and the sheep could see the food being replaced with fresh hay. Work has shown (previously and in this thesis) that sheep (and cattle) show an increased interest in fresh food even when they have had *ad-libitum* access (Winter & Hillerton, 1995; Sibbald, 1997), so it is possible that this increased interest may have compensated for any boredom/frustration arising from the protocol. Alternatively, this increased interest in fresh food may have increased the number of times the sheep were willing to pass through the door, exaggerating the lengths of time and amounts of food required to reduce the motivation to pass through the door. A method to identify whether the values obtained in chapter 7 are a fair reflection of these values could be to set up a series of doors in sequence. This would mean that the sheep would not be removed from a pen. Instead, after consuming a certain amount of food they would have to move into the next pen to continue feeding.

Of all the transport and food deprivation treatments the sheep were exposed to during the course of this thesis, being moved to an unfamiliar environment had the largest effect on post-treatment recovery (chapter 5). Post-treatment, the sheep that had been moved to unfamiliar environments did not show the increase in feeding behaviour that was seen in the other chapters. On its own, this result would lead to the conclusion that the deprivation during transport had not increased feeding motivation, as the sheep spent a lower proportion of scans feeding than at the equivalent time pre-treatment. The results from the other chapters show an increase in the motivation to feed following a period of food deprivation, which would suggest the experience of being moved has disrupted the usual pattern of behavioural responses.

The paddocks and sheds used in chapter 5 were on the same experimental site and it is likely that scents etc. were more similar than would be experienced in a commercial situation (for example, both types of housing were a similar distance from the on-site dairy). It could be that feeding would be reduced even further on a different site The implications of this reduction in feeding on the welfare of transported sheep depend on what happens to sheep at the end of their journey. If slaughtered soon after arrival at the destination, the fact that feeding is reduced in unfamiliar environments should not have a large impact compared with the manner in which they are handled. However, if the sheep are being held in lairage for any period of time (e.g. at markets, overnight at slaughterhouses) although the sheep may not readily consume any food that is provided, as a motivation to feed following periods of deprivation has been demonstrated, and this may cause welfare problems (by exposing animals to hunger) if not addressed.

As the push-door response required locomotion, the use of a treadmill may be a possible progression of the methodology developed here. This would take account of the increased foraging and number of steps taken that were observed during this work, by requiring the sheep to walk set distances for food rewards. The use of modern types of treadmills would also enable the work done to be controlled through the use of gradients. Dumont & Petit (1995) conducted a trial that was similar to the use of treadmills by designing a race sheep had to walk around to obtain limited amounts of food. However, that study examined the preferences for different quality hay after periods of food deprivation (rather than motivation alone), and demonstrated that food deprivation reduces the discrimination between good and poor quality hay by sheep. This would suggest an increased motivation to feed as they are taking the most freely available option. The use of a treadmill would indicate (as the push-door did) how much work sheep would be prepared to do in order to obtain food.

The results obtained still do not enable us to say if periods of food deprivation of around 24h in length result in something we could class as 'hunger', but the quite distinctive changes in behaviour seen after a period of food deprivation would

suggest some internal state driving the sheep to find food that could be considered to be associated with what we understand as 'hunger'. However, the fact that this change can be seen after only 6h would suggest that this may not cause suffering in the animals. The high cellulose content of a ruminants diet can lead to them spending around half of their day being spent feeding when at pasture (Lynch *et al.*, 1992) and a large proportion of the remaining hours are spent regurgitating this food and breaking it down further. Any large break from this cyclical pattern (especially if it coincides with the times of day when the sheep tend to do most of their feeding) is likely to disrupt the pattern of digestive behaviour causing a large effect on the behaviours performed.

8.2 Effect of transport

The vehicle used in this work was not a commercial sheep transporter. Others have done work on such vehicles (Knowles *et al.*, 1993; 1994; 1995; 1996), but the design of these vehicles limits the extent to which behavioural observations can be made. Using cameras set up in the transporters may be of use to confirm that the behaviours we have observed during transport are comparable with those performed in commercial conditions. Comments from field trials on the post-treatment behaviour of sheep state that they were primarily interested in feeding post-transport (Knowles *et al.*, 1994; 1996), which corresponds with the results found during this work.

The pre-treatment housing of sheep affected the response of sheep to transport, with increased standing in those loaded from fields compared with those loaded from a shed. This may suggest that sheep loaded directly from a field are more disturbed by the change in environment than those that have been moved inside prior to transport. Housing sheep inside pre-transport would give sheep experience of being confined in closer proximity to other sheep than when housed outside. Although sheep have been shown to take up to 9 weeks to become fully acclimatised to a novel environment (Fordham *et al.*, 1991), the experience of being confined may help them 'cope' with being confined on the vehicle.

One potential criticism of the transport experiments in this thesis (chapters 4, 5 and 6) is that the numbers were not large enough for statistical analysis. In particular, that the sheep were group-housed and therefore not independent. Work has shown that the individual housing of sheep for research purposes affects their behaviour (Marsden & Wood-Gush, 1986). If sheep had been individually housed for this work the results obtained would not necessarily be applicable to the responses of sheep in the commercial situation. In addition, sheep are a group-living species, their behaviour is influenced by that of others in the flock and this needs to be taken into account when assessing the effects of food deprivation and transport. The best solution to this problem would have been to increase the number of groups in each treatment, but this was not possible due to financial constraints. However, the agreement and similarity in the responses across chapters 4, 5 and 6 would suggest that the results can be considered valid. The responses also agree with those from chapter 2 (where sheep were housed in a suitable number of pairs to allow comparison of the pens rather than of the individuals) and previously published work (Cockram *et al.*, 1996) and observations from field trials (Knowles *et al.*, 1994; 1996).

8.3 Conclusions

A common theme throughout the experimental chapters is that that following a period of food deprivation (regardless of transport treatment) the first priority of sheep is to feed. The only factors investigated during this work that affected this response were changes in food and environment post-treatment. It would appear to be important to provide suitable food post-transport to satisfy the increased motivation demonstrated here. Whether or not the sheep could be considered to be feeling 'hunger' as experienced by humans is debatable, it is not possible to attribute such feelings from the type of work done here.

Feeding the sheep every 12h produced a similar result to that seen in cattle and sheep by other authors, with an increase in feeding behaviour with the presentation of fresh food. This increase may also have influenced the results of the motivation work in chapter 7, particularly during the experiment 3 where sheep were allowed to

feed for a limited time. They would have observed and/or perhaps smelled the fresh food that was placed in the reward pen after each successful pass through the push-door, which may increase the motivation to return to the reward pen. However, the results from control sheep using the push-door demonstrate that this does not occur, as even with the presentation of fresh food, sheep still failed to pass through the push-door.

A possible solution to the problem of dehydration caused by feeding conserved foods such as hay may be to provide only a limited amount of food post-treatment, sufficient to reduce the motivation to find food. This can be seen to result in a number of problems in itself (mainly the limited space that is generally available in lairages and at markets, as well as on vehicles). Ideally, sufficient food for all sheep to feed freely would be provided, however this may be unacceptable in slaughter-houses, who prefer to slaughter animals with emptier guts.

The work in this thesis demonstrated that the provision of a period of lairage off the vehicle reduced the effect of a 24h journey on liveweight, and reduced the length of time required for behaviour to return to pre-treatment levels. A 12h lairage had a greater beneficial effect than a 3h lairage in terms of post-treatment recovery. Leaving sheep on the vehicle during the lairage period can not be recommended as it had little beneficial effect on the post-treatment behaviour, and the sheep drank very little, which may have increased the risk of dehydration (compared with the 12h lairage).

Work has shown that following 9h and 14h journeys across the United Kingdom sheep required 24h for blood variables associated with short term stress (cortisol) and dehydration (osmolality) to recover to normal levels. For the recovery of metabolites associated with fasting (beta-hydroxybutyrate, urea, free fatty acids, liver glycogen), 96h were required for levels to be stabilised, but full recovery to normal levels has been reported to take up to 144h (Knowles *et al.*, 1993). The increase in recovery time (in terms of behavioural changes) in unfamiliar environments will also reduce any benefits of providing lairage (both in terms of recovery during lairage and post-treatment recovery), since current regulations only require the provision of a 1h rest period with access to water after 14h transport.

The current legislative requirement for lairage during journeys exceeding 8h can be seen to be of minimal benefit to sheep, as it is not long enough to allow any recovery of biochemical responses to the transport and associated food and water deprivation, but it is long enough to increase the risk of dehydration if food is provided. However, these conclusions are only in terms of the conditions experienced during this work, the elevated temperatures experienced in mainland Europe may alter the effects and should be investigated further. A controlled trial investigating the effect of temperature on the endocrine responses of sheep to food and water deprivation found sheep were able to maintain plasma osmolality during 48h food and water deprivation at 7°C and 35°C (Parrott *et al.*, 1996), which disagrees with field studies that have been performed by others who found changes in plasma osmolality after journeys to France lasting 18h and 24h, during which temperatures rose as the vehicles travelled to southern France (Knowles *et al.*, 1994).

It would be beneficial to undertake further work to further investigate the results found during the motivation work. Alternative methods could include weighting the food against another reward, such as company of another sheep or using the dominance hierarchy of a group to see how individuals react to the deprivation. The effect of food deprivation on the speed at which sheep passed through the push door and the number of times they were prepared to do so (chapter 7) and the increase in feeding post-transport would support the suggestion of an increased motivation.

Further developments of the push-door may also allow it to be used to assess other 'needs' and show us what is important to sheep in the context of modern agricultural and research practices rather than depending on what we feel/think/assume is important. One beneficial development of the push-door would be to replace the weights with a load cell, a system that has been used with poultry (Petherick & Rutter, 1990). This would require all sheep to exert the same total force before gaining access to the reward pen. In its current form, there was wide variation in how the sheep used the doors, the two independently moving doors enabled some sheep to gain access to the reward pen by only pushing against one door (provided they were small enough).

Changes in transport regulations are unlikely to overcome the large effects of a change in environment on the responses and post-transport recovery of sheep, as it is unrealistic to expect sheep to be provided with familiar environments post-transport. However, this is no reason not to try and minimise the effects of this change in environment on unloading. For example Provenza and others have been looking for methods of increasing the intake of novel foods. Provenza *et al.* (1995) found that food restriction did not increase the intake of novel foods, however familiarity of flavour alone has been shown to increase the intake of a novel food with the same flavour (Launchbaugh *et al.*, 1997). In addition, exposure to a number of novel foods in succession increased the acceptance of novel foods (Launchbaugh *et al.*, 1997). This may be a feasible way of minimising the effect of being given a novel food, by giving lambs access to a number of food types while on the farm.

8.4 References

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APPENDIX I: *Published Papers*

The following three papers have been published by the author

Jackson, R. E. & Waran, N. K. (1995). Problems in measuring feeding motivation in sheep. In: Proceedings of the 29th International Congress of the International Society for Applied Ethology (ed. S.M. Rutter, J. Rushen, H.D. Randle and J.C. Eddison), pp. 59-60. Universities Federation for Animal Welfare, Potters Bar.

Jackson, R. E., Cockram, M. S., Goddard, P. J., Doherty, O. M., McGilp, I. M. & Waran, N. K. (1999). The effects of 24h water deprivation when associated with some aspects of transportation on the behaviour and blood chemistry of sheep. *Animal Welfare:* (in press).

Jackson, R. E., Waran, N. K. & Cockram, M. S. (1999). Methods for measuring feeding motivation in sheep. *Animal Welfare 8:* 53-63.

The following papers have been published with the author as a contributing author

Cockram, M. S., Kent, J. E., Jackson, R. E., Goddard, P. J., Doherty, O. M., McGilp, I. M., Fox, A., Studdert-Kennedy, T. C., McConnell, T. I. & O'Riordan, T. (1997). Effect of lairage during 24h of transport on the behavioural and physiological responses of sheep. *Anim. Sci. 65:* 391-402.

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